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Common scab resistance in potato

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Abstract

Common scab disease of potato is distributed worldwide and not easily controlled. It is caused by *Streptomyces* species. The virulence mechanism of *Streptomyces* species is the production of thaxtomin A. Although the disease cycle of common scab of potato is well understood, the molecular processes underlying disease development and disease resistance are little known. The work performed in this thesis is a part of an on-going project on common scab resistance in potato, studying gene expression in response to *S. turgidiscabies* infection.

Transcription profiles established by RNA-sequencing indicate a number of putative defense-associated genes which uniquely expressed in resistant cultivar Beate at the initial stage of tuber development. Seven candidate genes of which were selected to investigate the correlation between their expression and common scab resistance in potato, using real-time PCR method. Apart from these seven genes, two candidate genes putatively encoding TXR1 proteins involving in thaxtomin A transport, two putative candidate auxin signaling-associated genes and a gene putatively encoding aminotransferase ALD1 were also investigated.

Comparisons of candidate gene expressions in resistant cultivars and susceptible cultivars indicate two selected candidate genes performed correlations between their expressions and common scab resistance, which are gene putatively encoding auxin binding protein 1 and gene putatively encoding aminotransferase ALD1. These findings putatively conceived of an idea that auxin signaling might promote common scab resistance in potato. On the other hand, the results are not in the agreement with the hypothesis that putative defense-associated genes which uniquely expressed in resistant cultivar Beate at the initial stage of tuber development might enhance common scab resistance.

Abbreviation

bp	base pairs
cDNA	complementary DNA
DNA	deoxyribonucleinacid
dNTP	deoxynucleoside triphosphate
mRNA	messenger RNA
PCR	polymerase chain reaction
RNA	ribonucleinacid
Rpm	rotations per minute

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1. INTRODUCTION

1.1 Common scab disease of potato

Potato, *Solanum tuberosum*, a member of the *Solanaceae* family, is an economically and socially important vegetable crop. According to the International Potato Center, potato is evaluated as the third most important food crop for more than a billion people's consumption in the world. In terms of food security, potato is considered as a critical crop for population growth in the next decades. For instance, potato is expected to meet 50% of the increased food demand in the next 20 years in China, the biggest potato consumer of the world.

Common scab disease of potato is distributed worldwide and causes economic losses in potato production. In Canada, economic losses caused by common scab were estimated from 15.3 to 17.3 million Canadian dollars, in 2002 (Hill & Lazarovits 2005). Similarly, economic losses caused by common scab in Tasmanian potato industry were estimated excess of 3.66 million Australian dollars, approximately 4% of total value of the Tasmanian potato industry (Wilson et al. 2009). Common scab disease also affects yield in extreme cases due to delayed growth and the increase in small tubers (Hiltunen et al. 2005; Wanner 2009). The economic loss is also due to the rejection of scabby tubers in fresh market and more required processes in steam peeling procedure to remove deeply pitted lesions (Loria et al. 1997; Wilson et al. 2010).

Common scab disease is characterized by scab-like lesions on the surface of potato tubers (Loria et al. 1997). Scab symptoms are commonly described as brown or dark brown corky lesions on the tuber periderm (Baang 1979), either superficial scab-like lesions on the surface (Loria et al. 2006), or deeply pitted or raised lesions (Wilson et al. 1999). Raised lesions can be discrete or coalesce to form large patches on the tuber surface. All types of lesions can be observed on tubers of the same plant (Keinath & Loria 1991).

1.2 Common scab-causing organisms

Common scab is a bacterial disease. Pathogens causing this disease in potato have been identified as a number of *Streptomyces* species. *Streptomyces* bacteria produce various naturally antibiotics, known as biologically active secondary metabolites (Loria et al. 2003).

More than 600 species of *Streptomyces* genus have been recorded, but only few have been identified as scab-causing pathogens, in which, *Streptomyces scabies* is predominant worldwide (Lambert & Loria 1989). Other common scab causal agents have been identified, such as *Streptomyces acidiscabies* (Lambert & Loria 1989; Loria et al. 2006), *Streptomyces turgidiscabies* found in Japan (Miyajima et al. 1998), in Finland (Kreuze et al. 1999) and in north America (Wanner 2009). *S. turgidiscabies* was first identified in Norway in a recent survey by Dees *et* al. In 29 different potato cultivars collected from 130 different fields, 31% of common scab lesions were found to be caused by *S. turgidiscabies*, whereas, 69% of common scab lesions were found to be caused by *S. europaeiscabiei.* Noticeably, *S. scabies* was not detected in Norway (Dees et al. 2012).

The *Streptomyces* genus, Gram-positive bacteria, is filamentous (Loria et al. 2006) and first characterized by the ability to produce melanin, a soluble brown pigment (Thaxter 1891). Common scab causing *Streptomyces* are soil-borne, survive as saprophytes and over-winter either on plant debris, in soil, or on the surface of tubers. They can also be seed-borne and transmitted by seed tubers (Wang & Lazarovits 2005; Wilson et al. 1999). *Streptomyces* species have an immobile life style and produce spores in chains by fragmenting aerial sporogenous hyphae for their dispersal (Loria et al. 2003). The inoculum is disseminated by water, wind, farm equipment carrying soil residue, or seed tuber (Wanner & Kirk 2015; Wharton et al. 2007).

1.3 Development of common scab disease

Only the developing underground parts of plant, such as stems, stolons and tubers are found susceptible to common scab disease (Wanner & Kirk 2015). Infection usually initiates with the onset of tuberization (Wharton et al. 2007). Between 3 and 20 days after the tuberization is crucial period for disease development (Khatri et al. 2010). Once the mature periderm is suberized, tubers become resistant to pathogenic *Streptomyces* (Wanner & Kirk 2015). When a spore contacts with a suitable host, it germinates and initiates the infection process (Wharton et al. 2007). *Streptomyces* species produce non-fragmenting substrate mycelium helping them to colonize and penetrate tubers in the soil. Mycelium dispenses in soil water and penetrates tubers via un-suberized lenticels, sites of gas exchange, and get through the periderm (Loria et al. 2006) or take advantage of open wound on the surface of tubers

(Wharton et al. 2007). After penetration, the pathogens occupy intercellular spaces and live on death tissues, and gain nutritions from organic compounds. Once pathogens pass through three peridermal cell layers, cell death occurs (Wharton et al. 2007). Then, corky cells are formed by healthy cells around the lesions and push the infected cells outwards. Once the outer layer is penetrated, a new layer is formed underneath. This cycle is repeated and scab lesions are formed and enlarged (Agrios 2005; Wharton et al. 2007). The disease cycle of common scab of potato caused by *Streptomyces* pathogen is illustrated in figure 1.

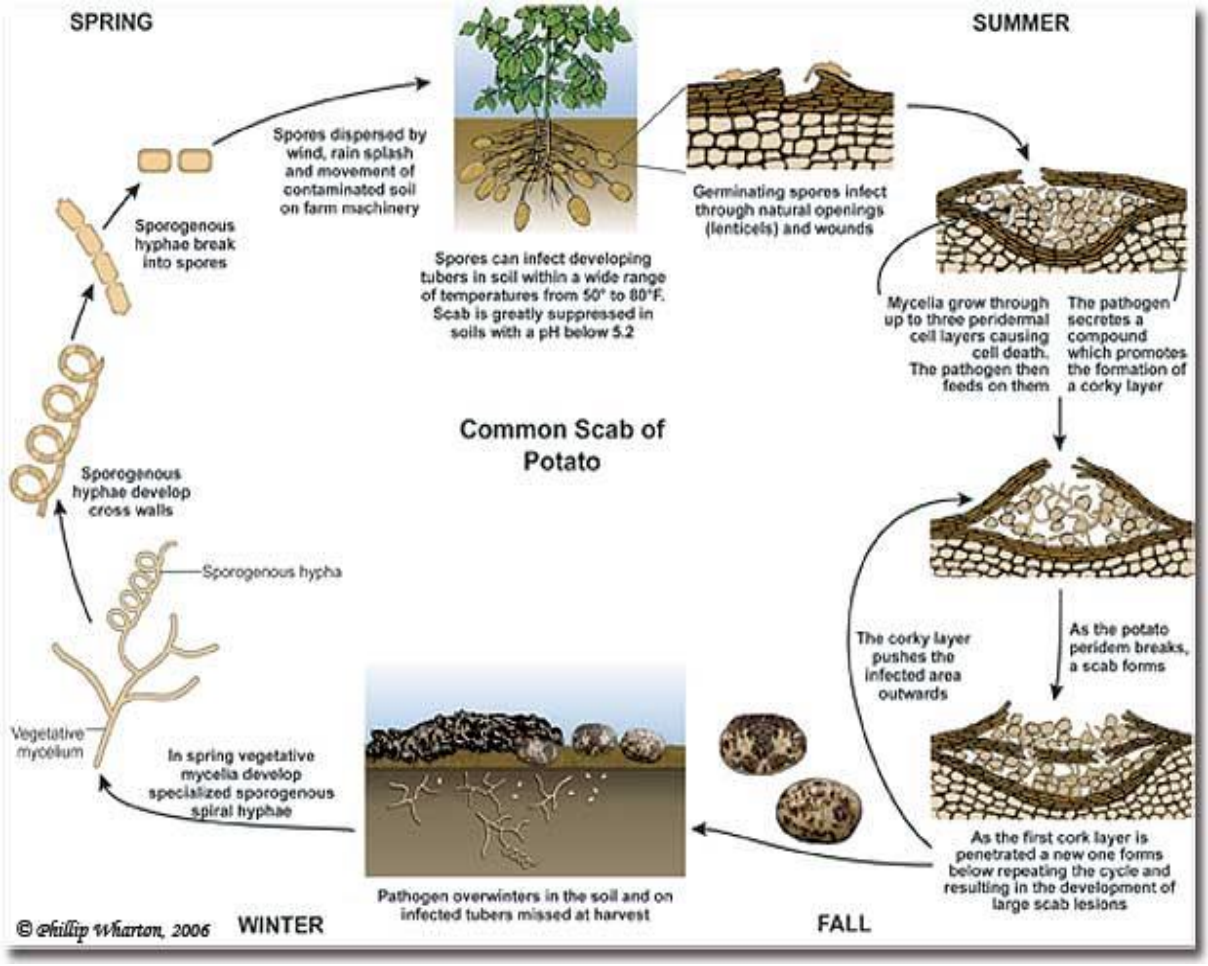


Figure 1: Disease cycle of common scab of potato caused by *Streptomyces* species. (The figure is taken from Wharton 2007).

Symptom development depends on the susceptibility of potato cultivar, pathogen virulence, pathogen density, and environment conditions. Scab is most developed when tubers grow

under warm, dry soil conditions with soil pH above 5.2 (Lambert & Loria 1989; Lazarovits et al. 2007; Wharton et al. 2007). The disease does not progress on tubers in storage (Khatri et al. 2010). Inoculum in infected seed tubers performed significantly increase in disease severity in daughter tubers and the significances of soil and seed tuber borne inoculum may vary upon environmental conditions, soil types, pathogen population and agronomic practice (Wilson et al. 1999). Moreover, a connection between the incidence of common scab, the level of seed tuber-borne inoculum and the population of pathogens surrounding root zone was also described by Wang and Lazarovits (2005).

1.4 Virulence mechanisms

To attack their host plants, pathogenic bacteria utilize various virulence strategies, such as productions of virulence proteins, plant hormones, or phytotoxins (Agrios 2005). Thaxtomin, a phytotoxin, was characterized as an important pathogenicity of common scab disease (Bignell et al. 2010; Healy et al. 2000). Its target is plant cell wall (Fry & Loria 2002). Thaxtomin A was found to induce common scab symptoms on aseptically cultured potato minitubers and plant generated tubers (King et al. 1991; Lawrence et al. 1990). The absence of genes related to thaxtomin biosynthetic in *S. acidiscabies* leaded to the abolishment of pathogenicity and no common scab occurred in potato (Healy et al. 2000).

There are four molecular virulence factors putatively causing common scab disease. The most important factor is the thaxtomin biosynthesis gene cluster, consisting of *txtA*, *txtB*, and *txtR*. *txtAB* genes encode for a peptide synthetase (Healy et al. 2000). *txtR* gene encodes for a binder of cellobiose derived from the host, which activates thaxtomin biosynthesis (Bignell et al. 2014a; Joshi et al. 2007). Another factor is *nos* gene, encoding nitric oxide synthase, which is required for the nitration of tryptophan, the first step in biosynthesis of thaxtomin (Kers et al. 2004). Two other molecular virulence factors are *nec1* gene conferring a necrosis protein and *tomA* gene encoding for an enzyme of saponinases (Bukhalid et al. 1998; Kers et al. 2005; Lerat et al. 2009). Recently, it was confirmed that *nec1* and *tomA* are not required for pathogenicity (Dees et al. 2012) and only genes related to thaxtomin production, such as *txtA, txtB, txtR* and *nos,* are required for common-scab pathogenicity (Dees et al. 2012; Seipke et al. 2011; Wanner & Haynes 2009).

Virulence related genes in pathogenic bacteria are usually located in pathogenicity islands (PAIs) in the genome (Hacker & Kaper 2000). PAI was indicated to be horizontally transferred into the genome of other species, thereby, confers pathogenicity to non-pathogenic species (Kers et al. 2005). This may explain the increasing range of pathogenic *Streptomyces* species (Loria et al. 2008). Genes related to thaxtomin production, such as *txtA, txtB,* and *txtR*, are located in 'toxicogenic region' of the first segment of the PAI (Lerat et al. 2009).

1.5 Thaxtomin A

1.5.1 Production of thaxtomin A

All common scab-causing streptomycetes have the same virulence mechanism that is production of thaxtomin (Bignell et al. 2014b). Thaxtomins, the phytotoxic secondary metabolites, are nitrated cyclic dipeptides derived from L-tryptophan and L-phenylanine (Healy et al. 2000; King et al. 1991). Production of thaxtomin by common scab-causing streptomycetes is only induced in living host tissues or in specific plant-based media containing oat grains or oat bran (Babcock et al. 1993; Loria et al. 1995).

Cellobiose and cellotriose, two cello-oligosaccharides from the host plant, were found to stimulate thaxtomin production in a defined medium. In which, cellobiose upregulated transcription of genes related to thaxtomin biosynthesis in scab-causing streptomycetes, whereas, cellotriose upregulated thaxtomin production (Johnson et al. 2007). In another research, cellobiose and cellotriose were also indicated as important factors regulating expression of *txtA, txtB,* and *txtR* genes in *S. scabies*. Particularly, cellobiose was indicated as a key to activate expression of *txtR* and initiate thaxtomin A production. Thus, cellobiose was considered to be a 'gatekeeper of *Streptomyces scabies* pathogenicity' (Francis et al. 2015).

1.5.2 Role of thaxtomin A in pathogenicity

Thaxtomin causes different changes in the host plants, such as cell hypertrophy, root and shoot stunting, tissue necrosis, inhibition of cellulose synthesis, alterations in plant Ca^{2+}, H^{+} ion fluxes, and programmed cell death (Duval et al. 2005; Errakhi et al. 2008; Fry & Loria 2002; Leiner et al. 1996; Lerat et al. 2009; Tegg et al. 2005).

In potato, thaxtomin A was found as a cellulose synthesis inhibitor and causing cell death. Electron micrographs of potato parenchyma cells treated with thaxtomin A (100µg/g tissue) described a detachment of plasmalemma cell wall after 4 hours (figure 2B), and irregular-shaped nucleus with lobes and invaginations after 24 hours (figure 2C) (Goyer et al. 2000).

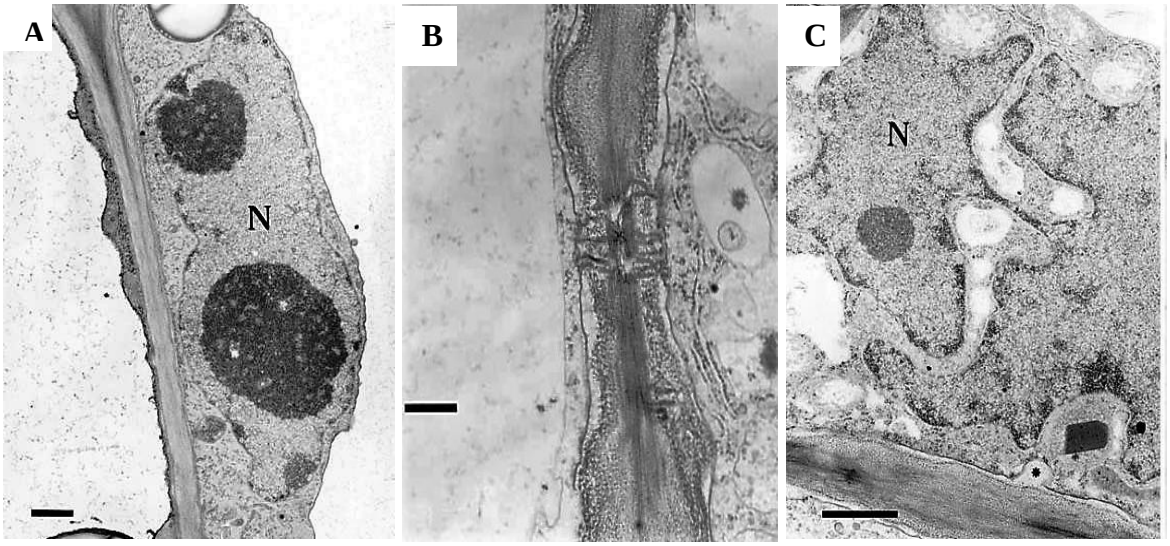


Figure 2: Electron micrographs of potato parenchyma cells treated with thaxtomin A. (**A**) Parenchyma cell observed after 12 hours without thaxtomin A treatment, with normal cell wall and regular-shaped nucleus (N), with smooth contour. Scale bars = 1µm. (**B**) Detachment of plasmalemma in parenchyma cell from cell wall observed after 4 hours treated with thaxtomin A 100µg. Scale bars = 0.5µm. (**C**) Irregular-shaped nucleus of the parenchyma cell, with lobes and invaginations, observed after 24 hours. Scale bars = 1µm. (Pictures A, B, C are adapted from Goyer et al. 2000).

Thaxtomin A was also indicated to induce programmed cell death in potato and other plants. Experiment on mature potato tuber discs inoculated with *S. scabies* showed tissues turned brown (Burrell 1984). Similarly, electron micrographs of potato parenchyma cells treated with thaxtomin A (100µg/g tissue) described a disappearance of all cell organelles in the cytoplasm and a rupture and distortion of cell wall after 72 hours (Goyer et al. 2000).

In radish, thaxtomin A was found to reduce shoot and root length, to cause stunting and hypertrophy of seedlings or tissue chlorosis and necrosis at concentrations below 1.0 µM (Loria et al. 1997). In the root of *Arabidopsis thaliana* and pollen tube tissue of tomato, a rapid Ca^{2+} influx and H^{+} efflux across the plasma membrane were observed within a minute after application of thaxtomin A (Tegg et al. 2005). Similarly, in *Arabidopsis*, thaxtomin A

was found to induce an increase in anion current across the plasma membrane and Ca^{2+} influx, subsequently increased vacuolar pH and resulted in cell death (Errakhi et al. 2008).

1.5.3 Transport of thaxtomin A

The transport of thaxtomin A within plant cells of *Arabidopsis* was indicated to be associated with a novel protein encoded by *TXR1* gene. Function of *TXR1* gene was characterized by a point mutation at stop codon of this gene in a thaxtomin A-resistant *Arabidopsis*. Sequence of thaxtomin A-susceptible wild type *TXR1* indicated the stop codon is at amino acid 116, whereas, sequence of mutated *txr1* gene in thaxtomin A-resistance *Arabidopsis* indicated the stop codon at amino acid 98. The difference in lengths of these two genes did not lead to difference in transcriptions, but it contributed to different functions in the transport of thaxtomin A (Scheible et al. 2003).

1.6 Plant-pathogen recognitions

Many molecular interactions are induced during the infection in both host plant and pathogen. Pathogens are generally able to manipulate biochemical and physiological processes in the hosts to release molecular compounds known as pathogen elicitors (Agrios 2005). As soon as the plant receives signal molecules indicating the presence of a pathogen, it will also synthesize an arsenal of defense-related compounds to combat invaders. A specific interaction between pathogen elicitor and corresponding plant disease receptor is thus activated (Agrios 2005). When pathogen elicitors are recognized by plant disease receptors, a cascade of specific defense responses will be initiated in the plant. Alternatively, in susceptible host, pathogen elicitors are not recognized by plant disease receptors, and thereby, pathogen elicitors will be able to overcome plant's unspecific defense responses and promote infection (Dangl & Jones 2001).

Most recently, it was indicated that *S. scabies* recognizes the host plant by the cellobiose sensor CebR (Francis et al. 2015). CebR is a master regulator of cellulose and cello-oligosaccharide catabolism (Marushima et al. 2009). CebR recognizes the host via the interaction with cellobiose produced by the host, then, activates expression of virulence-related genes and subsequently produces thaxtomin A (Francis et al. 2015). This study also suggested that CebR is the most likely important regulator of the virulence in *S.*

turgidiscabies and *S. acidiscabies.* In another study on plant-pathogen interactions in *Streptomyces* species, it was also indicated that the recognition of cellobiose by *Streptomyces* species induces production of diffusible nitric oxide (NO) at the host-pathogen interface in response to cellobiose. Thus, NO produced by *Streptomyces* was suggested to play an important role in the host-pathogen interaction (Johnson et al. 2008).

Thaxtomin A was revealed to create a rapid Ca^{2+} influx in plants cells (Errakhi et al. 2008; Tegg et al. 2005). Ca^{2+} dependent protein kinases (CDPKs) was found to have a function of Ca^{2+} sensor in response to increase in cytosolic Ca^{2+}, which activates plant defense system through other signaling transduction pathways for cell wall defenses in potato (Kobayashi et al. 2007). In *Arabidopsis*, genetic studies also revealed pivotal role of plant cyclic nucleotide gated ion channel proteins (CNGCs) in response to pathogen signals by providing a pathway for Ca^{2+} conductance across the plasma membrane (Ali et al. 2007; Leng et al. 2002; Ma et al. 2010; Ma & Berkowitz 2011; Qi et al. 2010).

1.7 Plant defense mechanisms

In general, plants defend themselves against pathogens by a combination of several defense strategies. The first line of host defense is altering its surface, which functions as structural barriers to inhibit pathogen' invasion and spreading throughout the plant, for instance, the boundary formed by the rigid cellulose outer leaf cuticle cells or lignin polymerization in plant secondary cell walls (Agrios 2005; Taiz & Zeiger 2010). Another strategy is biochemical reaction to produce either toxic substances to eliminate the pathogen or substances, such as antimicrobial and antifungal compounds, to suppress pathogen's growth (Heath 2001; Hueckelhoven 2007), or phytoalexins (Hueckelhoven 2007) or disease resistant proteins (Hara-Nishimura & Hatsugai 2011; Taiz & Zeiger 2010). Programmed cell death is also an important defense strategy, where infected cells are killed in order to prevent the pathogen from spreading within healthy cells (Hara-Nishimura & Hatsugai 2011).

Since the target of thaxtomin A is cell wall, a number of studies about plant cell wall-associated defense, mainly on *Arabidopsis*, have been reported (Bischoff et al. 2009; Duval & Beaudoin 2009). Although a Ca^{2+} influx was observed as a typical early signal inducing defense response (Errakhi et al. 2008; Tegg et al. 2005), the molecular processes underlying disease defense responses and resistance to common scab of potato are still insufficiently

understood (Wanner & Kirk 2015). No typical defense-response gene was found in a cDNA library from common scab disease tubers (Flinn et al. 2005).

1.7.1 Suberization

Suberization is known to help the host plant to overcome pathogen's invasion by setting tuber skin and closing the lenticels (Khatri et al. 2011). Suberin is a key compound in regulating transpirations in potato tuber periderm, an important external barriers in tubers protecting the fleshy parenchyma from water loss and pathogen penetration (Taiz & Zeiger 2010). In potato tuber, the periderm is constituted of three layers: the phellem or cork layer, the phellogen or mother layer, and the phelloderm (Lulai & Freeman 2001; Peterson & Barker 1979). Externally, the dividing phellogen can build up 6-12 layers of death cells with suberized walls to form impermeable phellem or cork layer. Internally, the dividing phellogen builds up a few layers of parenchyma cells with cellulosic wall, known as the phelloderm, connecting the periderm to tuber flesh (Sabba & Lulai 2002). During phellogen dividing, this layer is physiologically active with thin and fragile cell wall. After phellogen stops dividing, it is inactive with thickened cell walls and cork layer embedded to phelloderm layer. This process is known as skin set occurring within 14-21 days of maturation period (Lulai & Freeman 2001).

Recently, a study on common scab disease of potato reported that *S. scabies* induces phellem suberization within 7 days after infection, 28 % greater than the negative control. Thicker phellem suberization, up to 8 cell layers, was also observed in resistant cultivar Russet Burbank, between 10 to 30 days after tuberization, whereas, only 6 cell layers were observed in susceptible cultivar Desiree (Khatri et al. 2011).

1.7.2 Apoplastic defenses - Phytoalexins

Apoplast is the intercellular air space in the continuous system, allowing water transport through cell wall and extracellular space (Taiz & Zeiger 2010). After the recognition of the pathogen, the plant induces apoplastic defenses to protect the cell wall from pathogen's penetration. Plants can utilize several ways to control pathogenic intruders, consisting of suppressing microbial cell wall-degrading enzymes, structural and chemical remodeling the

cell wall at the sites of attack, and killing pathogens by antimicrobial compounds (Tanaka et al. 2006; Zhang et al. 2004).

The most important plant antimicrobial compounds are phytoalexins. Phytoalexins play a crucial role in plant resistance involved in inhibiting pathogen development and cell wall strengthening (Hueckelhoven 2007). In potato, four main classes of phytoalexins have been characterized, consisting of the phenylpropanoid-related phytoalexins: caffeic acid and chlorogenic acid (Jeandet et al. 2013; Kuc 1957); the steroid glycoalkaloids: α-solanine and α-chaconine (Jeandet et al. 2013; Kuc 1984); the norsesquiterpenoids: sesquiterpenoids and rishitin (Taiz & Zeiger 2010; Tomiyama et al. 1968), and the coumarin, scopoletin (Jeandet et al. 2013; Taiz & Zeiger 2010).

Noticeably, phenolic acid contents in red-skinned potato cultivars were indicated to be positively correlated to common scab resistance caused by *Streptomyces* species in very early stage of the disease development (Burrell 1984; Singhai et al. 2011). In barley, cell wall-bound phenolics were also found to be locally accumulated to prevent penetration of *Erysiphe graminis f. sp. Hordei* causing powdery mildew. However, it is unknown whether phenolic functions as a poison agent or as cell wall strengthening principle (Von Ropenack et al. 1998).

1.7.3 Plant cell wall- associated defenses

1.7.3.1 Cell wall thickening

After cell expansion ceases, in some cases, cells continue to synthesize a thick secondary cell wall for mechanical support (Taiz & Zeiger 2010). In a genetic research on transcription profiles of mRNA isolated from *Arabidopsis* suspension cells subjected to thaxtomin A (2µM), it was putatively implicated that thaxtomin A induces expression of genes related to strengthening the cell wall and reinforcement of cell wall, such as pectinesterase (10.05-fold upregulation), and xyloglucan endotransglycosylase (8.44-fold upregulation) (Duval & Beaudoin 2009).

Another cell wall thickening mechanism is lignification. Lignin is one of an important cell wall components in various cell types to support and conduct tissues. First, lignification makes the cell wall more resistant to the mechanical pressure during pathogen penetration

(Taiz & Zeiger 2010). It also protects the cell from cell wall-degrading enzymes because lignified cell wall is water resistant (Bechinger et al. 1999). Lignin is derived from phenylalanine. Phenylalanine ammonia-lyase (PAL) is the first enzyme catalyze phenylalanine to form lignin precursors (Taiz & Zeiger 2010). PAL was found 2.92-fold upregulated when *Arabidopsis* suspension cells subjected to thaxtomin A (2µM) (Duval & Beaudoin 2009).

In addition, cellulose microfibrils are known to strengthen cell wall as cellulose microfibrils are crystalline ribbons, which are relatively resistant to enzymatic degradation (Taiz & Zeiger 2010).

In potato, when parenchyma cells were subjected to thaxtomin A (100µg), fibrillary material was microscopically observed in the intercellular space after 12 hours (figure 3) (Goyer et al. 2000).

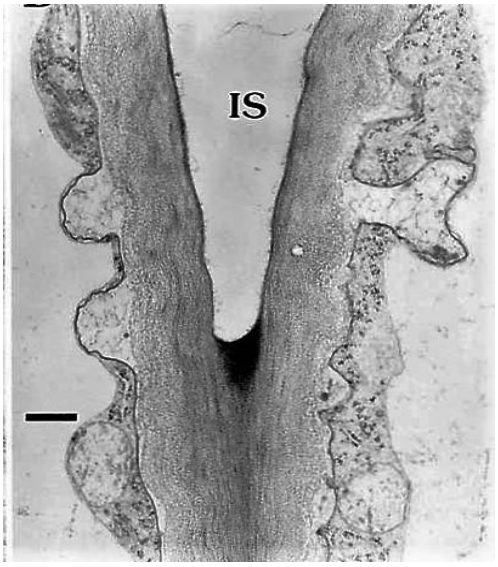


Figure 3: Electron micrographs of potato parenchyma cells. Fibrillary material formed in intercellular space (IS) after 12h treated with thaxtomin A 100µg. Scale bar = 0.5 µm. (The picture is adapted from Goyer et al. 2000).

1.7.3.2 Plant cell wall strengthening - Auxin signaling in plant disease resistance

Although definitive evidence for the role of auxin signaling in cell wall strengthening in response to common scab of potato has not been achieved, the exogenous application of auxin via foliar spray was indicated to induce resistance to common scab of potato caused by *S. scabies* and to inhibit thaxtomin A toxicity (Tegg et al. 2008). In addition, the expression of a number of auxin-related genes, including auxin homeostasis, auxin transport and auxin signaling were reported to be regulated during the infection process in *Medicago truncatula* caused by fungal pathogen *Macrophomina phaseolina*. The differential expression patterns of these auxin-related genes suggested that auxin signaling might play a potential role in the plant defense response against *M. phaseolina* (Mah et al. 2012).

Auxin signaling has also been suggested to be involved in plant disease resistance in some other studies on rice (Ding et al. 2008; Fu et al. 2011; Sauer et al. 2013). The role of auxin signaling pathway in disease resistance is to suppress expression of expansin genes which loosen cell wall, by conjugating assess auxin to amino acids. Thereby, auxin conjugation activity contributes to cell wall strengthening to prevent pathogen penetration (McQueenmason & Cosgrove 1995). Auxin conjugation depends on expression of auxin-inducible *GH3* gene, encoding IAA-amido synthetase. However, expression of *GH3* gene is repressed by AUX/IAA repressor protein when it binds to auxin response factor (ARF) and inactivates the transcription of auxin- inducible gene *GH3.* Therefore, auxin conjugation activity is only activated when AUX/IAA repressor protein is degraded (Leyser 2006; Taiz & Zeiger 2010).

AUX/IAA repressor protein is a part of auxin receptor located in the nucleus. It is unstable and can be degraded by ubiquitin-proteasome pathway when auxin concentration is elevated. The other part of auxin receptor is the SCF^{TIR1} complex. To degrade AUX/IAA repressor, auxin is required to bring AUX/IAA repressor and the SCF^{TIR1} complex together and activate auxin receptor. Activated auxin receptor will then ubiquitinate AUX/IAA repressor, leading to the degradation of AUX/IAA repressor by 26S proteasome. Subsequently, expression of auxin-inducible *GH3* gene is activated to initiate auxin conjugation activity (Leyser 2006; Meng et al. 2008; Taiz & Zeiger 2010; Tan et al. 2007). The regulation of auxin-inducible *GH3* gene expression by auxin is demonstrated in figure 4.

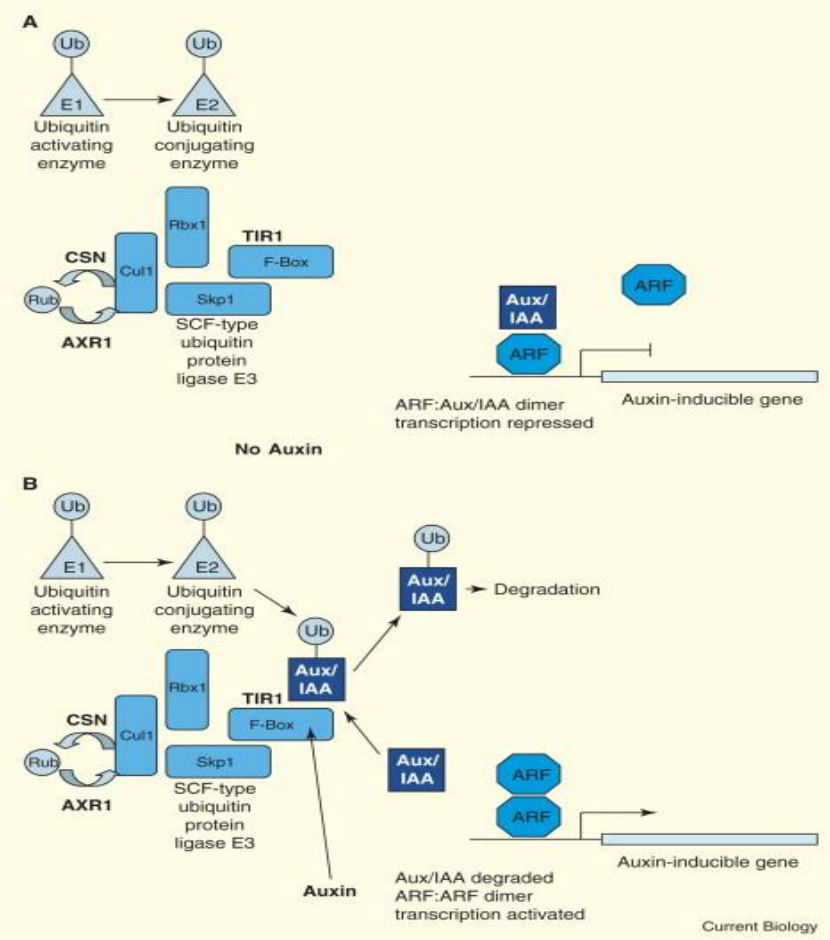


Figure 4: Regulation of expression of auxin-inducible gene by auxin. (The figure is taken from Leyser 2006).

Ubiquitination pathway is initiated by the catalysis of ubiquitin-activating enzyme E1 which requires ATP. E1 then transfers ubiquitin to the ubiquitin-conjugating enzyme E2. Ubiquitin E2 is subsequently transferred to the ubiquitin-protein ligase E3, which transfers ubiquitin to targeted AUX/IAA repressor protein. E3 ligase complex, known as The SCF^{TIR1} complex, is composed of 4 components: TIR1 (transport inhibitor response 1), a specific E3 ubiquitin ligase complex functioning as auxin F-box binding protein; SKP1 (Apoptosis signal-regulating kinase 1); RBX1 (ring-box 1); and CUL1 (cullin 1). The ubiquitinated AUX/IAA protein is finally targeted to the 26S proteasome and degraded (Taiz & Zeiger 2010). Factor required for efficient activity is Rub1 involved in addition of the ubiquitin-like protein to the Cullin subunit (AXR1) and removal of the Cop9 signalosome (CSN) from the Cullin subunit (Leyser 2006). (**A**) Without auxin, the dimerization of AUX/IAA repressor protein and auxin response factor (ARF) blocks the expression of auxin-inducible gene. (**B**) Auxin brings the F-box protein and AUX/IAA repressor protein together, then promotes the degradation of AUX/IAA repressor protein through ubiquitin-proteasome pathway, leading to the release of ARF to promote the transcription of auxin-inducible gene (Leyser 2006).

1.7.4 Programmed cell death

Another plant defense mechanism is the hypersensitive reaction (HR), leading to programmed cell death (PCD). PCD is the plant hypersensitive immune response to pathogen, in which, infected the cell is killed through programmed mechanisms in the plant cell to eliminate the pathogen or to prevent them from integrating into new healthy cells (Fukuda 2000). PCD is induced in response to the plant sensing a pathogen-associated molecular pattern (PAMP) molecules. These PAMP molecules are recognized by pattern recognition receptor (PPR) proteins, located on the plasma membrane. An increase of cytosolic Ca^{2+} is created early in the signaling cascade in response to PAMP molecules sensed by plasma membrane PPR proteins and immune responses are subsequently activated. Typical features of PCD are the production of reactive oxygen species (ROS) such as H_2O_2 and NO; the activation of the jasmonate or salicylic acid pathways; and protein kinases (Ma & Berkowitz 2011). In response to increased cytosolic Ca^{2+}, Ca^{2+} dependent protein kinases (CDPKs) have function as Ca^{2+} sensors, and act in the downstream.

In potato, these CDPKs were found to generate the (ROS) through NADPH oxidase, in response to Ca^{2+}. Increased cytosolic Ca^{2+} was found to activate plant defense systems against pathogens via other signaling pathways (Kobayashi et al. 2007). In addition, CDPKs was determined to regulate gene expression in response to increased cytosolic Ca^{2+} induced by pathogen (Ma & Berkowitz 2011).

1.7.4.1 Roles of vacuoles in programmed cell death

Vacuoles play important roles in defense mechanisms in programmed cell death. In most of mature plant cells, vacuoles occupy up to 90% of the cell volume (Taiz & Zeiger 2010). According to their functions, vacuoles are characterized as two types with opposite functions. The first type is lytic vacuole containing hydrolytic enzymes required for non-selective digestion of cell contents during PCD such as aspartate proteinases (Taiz & Zeiger 2010; van der Hoorn 2008), cysteine proteinases (Yamada et al. 2001), DNase, RNase and nucleases (Obara et al. 2001; Taiz & Zeiger 2010). The second type is protein storage vacuole containing disease resistance proteins against invading pathogens (Neuhaus et al. 1991) myrosinases (Ueda et al. 2006), or toxic proteins (Yamada et al. 1999) and lectins (Bednarek & Raikhel 1991). Vacuole-mediated cell death occurs in two different ways: non-

destructive way in the fusion of the plasma membrane and the vacuolar membrane (Hatsugai et al. 2009); and destructive way in the vacuolar membrane collapse (Kuriyama & Fukuda 2002).

In potato, cell death caused by thaxtomin A was microscopically observed after 72 hours subjected to thaxtomin A (100µg) with the disappearance of cell organelles and ruptured cell wall (figure 5) (Goyer et al. 2000).

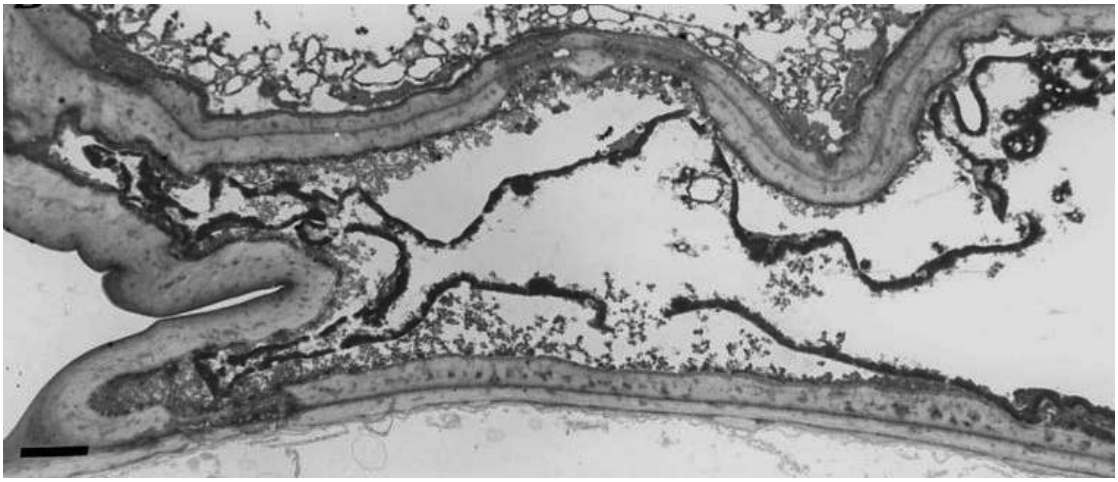


Figure 5: Electron micrographs of potato cell death observed after 72 hours of thaxtomin A (100µg) treatment. Parenchyma cell was observed with distorted and ruptured cell wall and unidentified cell organelles. Scale bar = 2µm. (Picture is adapted from figure 4, Goyer et al. 2000).

In another study, epifluorescent microscopic observation also demonstrated that nucleus, vacuole, many active mitochondria and chloroplasts suddenly disappeared only several hours after formation of cell wall thickening (Groover et al. 1997). It was suggested that vacuolar collapse was caused by an increase in Ca^{2+} influx through plasma membrane leading to an increase in valuolar pH (Groover & Jones 1999). In potato, thaxtomin A was reported to create an increase in Ca^{2+} influx through plasma membrane (Errakhi et al. 2008; Tegg et al. 2005), distorted cell wall and disappearance of cell contents (Fry & Loria 2002; Goyer et al. 2000). All of these activities are related to vacuole collapse and consequently destructive cell death, which is eventually remained as the hollow cell with thickened cell wall (Groover et al. 1997; Groover & Jones 1999; Hara-Nishimura et al. 2005; Hara-Nishimura & Hatsugai 2011).

1.7.4.2 Disease resistance proteins – Non-destructive programmed cell death

Disease resistance proteins, are usually toxic and not allowed to release into the cytosol (Taiz & Zeiger 2010; Yamada et al. 1999). Therefore, they are usually synthesized on the endoplasmic reticulum as larger precursors and then transported into protein storage vacuoles, where precursor proteins are converted into their respective mature forms by vacuolar processing enzyme (Hara-Nishimura & Hatsugai 2011; Taiz & Zeiger 2010). Bacterial infection triggers membrane fusion by proteasome, in which, plasma membrane and tonoplast are fused together to discharge disease resistance proteins into apoplast, where bacteria live and proliferate, contributing to suppression of pathogen proliferation (Hara-Nishimura et al. 2005; Pajerowska-Mukhtar & Dong 2009). In this case, non-destructive PCD is caused by proteasome-mediated membrane fusion in defense against bacterial infection (Pajerowska-Mukhtar & Dong 2009), thus causes local necrosis (Muthamilarasan & Prasad 2013).

Most recently, in order to identify candidate genes for common scab resistance, a research on potato gene expression in responses to *S. turgidiscabies* was carried out in susceptible potato cultivar Saturna and relatively resistant cultivar Beate. RNA-sequencing method was used to obtain transcription profiles at elongating stolon stage (early hook stage) and swelling stolon stage (early tuber formation stage). A number of genes putatively encoding for disease resistance proteins were suggested to be involved in common scab resistance since they uniquely expressed in relative resistant cultivar Beate, such as at4g27190 belonging to intracellular nucleotide binding-leucine-rich repeat (NB-LRR) family (Dees et al. 2015; Mayer et al. 1999), RPP13 providing resistance to downy mildew in *Arabidopsis thaliana* (Bittner-Eddy & Beynon 2001; Dees et al. 2015), rga3 resistance protein and late blight resistance proteins (Dees et al. 2015).

1.7.5 Detoxification of thaxtomin A toxicity

Glucosylation was described as the detoxification of thaxtomin A toxicity by Acuña et al. (2001). Principle of glucosylation is glucose conjugation, which is mediated by glucosyltransferase enzyme, transferring glucose from UDP-glucose to free -OH groups. In Acuña et al. research, thaxtomin A-β-di-*O*-glucoside from potato minitubers of common

scab-resistant cultivar Nooksack and common scab-susceptible cultivar Ranger was isolated and characterized as the glucose conjugate of thaxtomin A. Noticeably, thaxtomin A-β-di-*O*-glucoside was found six time less toxic than thaxtomin A in potato minituber slice tests. In addition, glucosyltransferase enzyme level was found almost double in resistant cultivar Nooksack than that in susceptible cultivar Ranger. It was suggested that the function of glucosyltransferase in potato tubers might be related to resistance to common scab disease because of the detoxification of thaxtomin A toxicity (Acuña et al. 2001). Similarly, thaxtomin A and B was also found partially transformed into thaxtomin A-*β*-D-glucoside and thaxtomin B-*β*-Dglucoside by *Bacillus mycoides* cultured in oatmeal broth. (King et al. 2000).

In addition, a correlation between sugar contents in potato tuber peel and common scab disease was indicated. Contents of glucose, fructose and sucrose in the peel of *S. scabies*-causing scabby tubers from six potato cultivars were found lower than those in scab-free tuber peel. Similarly, glucose and fructose contents in common scab-resistant cultivars were found higher than those in susceptible ones (Goto 1981).

1.8 Controls of common scab disease

Common scab disease of potato is not easily controlled and no single control methods are reliable except the use of resistant potato cultivars (Wanner & Kirk 2015). Nevertheless, the disease can be effectively controlled by an integrated approach, which combines the use of resistant host, reduction of pathogen densities, and management of growing conditions (Wanner & Kirk 2015; Wharton et al. 2007).

1.8.1 Resistant potato cultivars - Breeding for resistance

To score common scab susceptibility is challenging as it largely varies in symptom scores, ranging from no common scab symptoms to severe pits among tubers from the same plant (Driscoll et al. 2009). Cultivar Russet Burbank, one of the most prevalent cultivar in the world, was indicated moderately resistant to the disease, however, it is still susceptible to deep lesions under highly pathogen inoculum densities (Wilson et al. 2010). In Finland, cultivar Bellona and cultivar Matilda performed higher disease incidence and more severe common scab symptoms caused by *S. scabies* and *S. turgidiscabies* than cultivar Sabina

(Hiltunen et al. 2005). In Norway, cultivar Beate was found to be relatively resistant to *S. turgidiscabies,* and cultivar Saturna was found to be susceptible to *S. turgidiscabies* (Dees et al. 2015). Whereas, according to the European Cultivated Potato Database (www.europotato.org/), both cultivar Beate and cultivar Saturna are resistant to *S. scabies.*

Although no completely resistant cultivars have been achieved, resistant cultivar is the most reliable method (Wanner & Kirk 2015). To create common scab-resistance potato cultivars has been the desired goal of many potato-breeding programs (Haynes, K. G. et al. 2009; Loria et al. 2006). It was determined that common scab resistance is quite simply inherited when common scab-resistant diploid potato, with ability to produce 2n male gametes, was crossed with common scab-susceptible tetraploid female parent (Murphy et al. 1995). Similarly, breeding within diploid *Solanum phureja-Solanum stenotomum* population was indicated to be feasible to transfer common scab resistance in this population to the tetraploid level via 4x-2x crosses (Haynes, K. et al. 2009; Murphy et al. 1995).

Thaxtomin A tolerance has been commonly used as a screening factor to figure out common scab-resistant potato lines for breeding (Hiltunen et al. 2006). Somatic cell selection in potato applying thaxtomin A resistance as a positive selection has been used by Hiltunen et al. (2011) and Wilson et al. (2010). However, in another study, Tegg & Wilson (2010) discovered that although thaxtomoin A-tolerance is a useful tool for screening resistance lines, some common scab-susceptible cultivars are also tolerant to thaxtomin A and conversely, some common scab-resistant cultivars were found sensitive to thaxtomin A. This finding was discussed as a reason why potato breeding for common scab-resistance remains elusive (Tegg & Wilson 2010).

Naturally occurring or induced mutations have been used in breeding programs when the mutations are stable and beneficial (Waterer et al. 2011). In 2004, new lines of red skinned potato cultivar Red Norland were achieved from naturally occurring mutations of three Red Norland tubers performed zones of darker skin colors. Sprouts taken from both light and dark colored zones of these tubers were vegetative propagated and grown for several generations. It was indicated that the change in skin color obtained from chimera mutation was stable in color changes. Interestingly, lines with superior red skin performed enhancement in common scab resistance. Therefore, this study suggested that chimeras can

be represented a rapid, low-cost method for improvement of red skin color and common scab resistance in potato (Waterer et al. 2011).

1.8.2 Reduction of pathogen densities - Seed tuber and soil treatments

1.8.2.1 The uses of fungicides, soil additives and soil fumigants

Several studies found that seed-tuber inoculum can increase disease severity and the level of inoculum on the seed tuber can be reduced by seed tuber treatments (Wang & Lazarovits 2005; Wilson et al. 1999). A number of fungicides have been applied on seed tuber surface as either dusts or sprays before planting, such as fluazinam, fenpiclonil, flusulfamide, and mancozeb (Wilson et al. 1999). The herbicide 2,4 dichlophenoxyacetic acid (2,4-D) has been shown to suppress the development of common scab in potatoes (Thompson et al. 2014).

Most recently, Al-Mughrabi et al. (2016) revealed that common scab incidence could be reduced 35.4% by using fludioxonil seed treatment, 36% by adding mustard meal to the soil, 30.0% by fumigating soil with Pic-Plus and 27.2% by fumigating soil with chloropicrin. Potential alternative for common scab management was also described in seed-tuber treatment with biopesticide containing *Bacillus subtilis*, which significantly reduces common scab disease of potato (Al-Mughrabi et al. 2016).

1.8.2.2 Biological controls

Non-pathogenic *Streptomyces* strains, EF-50 and EF-73, were found significantly reduce common scab incidence, up to 40%, because they utilize thaxtomin A as carbon and nitrogen sources (Doumbou et al. 1998). Likewise, two *Streptomyces* strains, *S. scabies* PonR and *S. diastatochromogenes* PonSSII, were discovered antagonistic towards pathogenic *S. scabies* and potential for biological controls in common scab disease (Liu et al. 1995; Liu et al. 1996). In addition, *Streptomyces* isolate WoRs-501 was detected to suppress scab-causing *Streptomyces* populations in the soil (Kobayashi et al. 2015). *Pseudomonas* BUM 223 was indicated to significantly suppress the growth of *S. scabies*, inhibit expression of genes *txtA* and *txtC* involved in thaxtomin A biosynthesis (St-Onge et al. 2011).

1.8.3 Managements of growing conditions

High soil moisture during tuberization dramatically reduces common scab in potato (Wanner & Kirk 2015; Wharton et al. 2007). Similarly, in Norway, increased irrigation during the stolon elongation and during tuberization was indicated to reduce common scab symptoms caused by *S. europaeiscabiei* and *S. turgidiscabies* (Johansen et al. 2015).

Acidic soils with pH of 5.0 to 5.2 are commonly applied for common scab control in potato (Wharton et al. 2007). In North West Tasmania, common scab symptoms were found reduced in soils with pH below 5.0 – 5.2 (Lacey & Wilson 2001). Contrastingly, common scab symptoms were found significantly reduced in alkaline soils with pH above 8.5 (Waterer 2002).

1.9 The aim of thesis

Although the disease cycle of common scab of potato is well understood, the molecular processes underlying disease development and disease resistance are still little known. To broaden understandings of infection process and to determine putative resistance-related genes, an on-going project on common scab resistance in potato, studying gene expression in response to *S. turgidiscabies* infection, has been carried out at the Department of Biotechnology and Molecular Genetics, Norwegian Institute of Bio-economy Research (NIBIO). Transcription profiles of the resistant cultivar Beate and the susceptible cultivar Saturna at two developmental stages, the early hook stage and the early tuber formation stage, were established by RNA-sequencing. The transcription profiles determine a number of putative defense-associated genes which uniquely expressed in resistant cultivar Beate at the early hook stage (Dees et al. 2015).

This thesis is a part of this on-going project at NIBIO, aimed to study expression of a number of putative defense-associated genes during infection with *S. turgidiscabies*, which are putatively suggested by Dees et al. (2015). To investigate the correlation between the expression of the candidate genes and common scab disease resistance, we compared the expression of candidate genes by real-time PCR method in several resistant cultivars and several susceptible cultivars, including cultivar Beate and cultivar Saturna. In which, mRNA

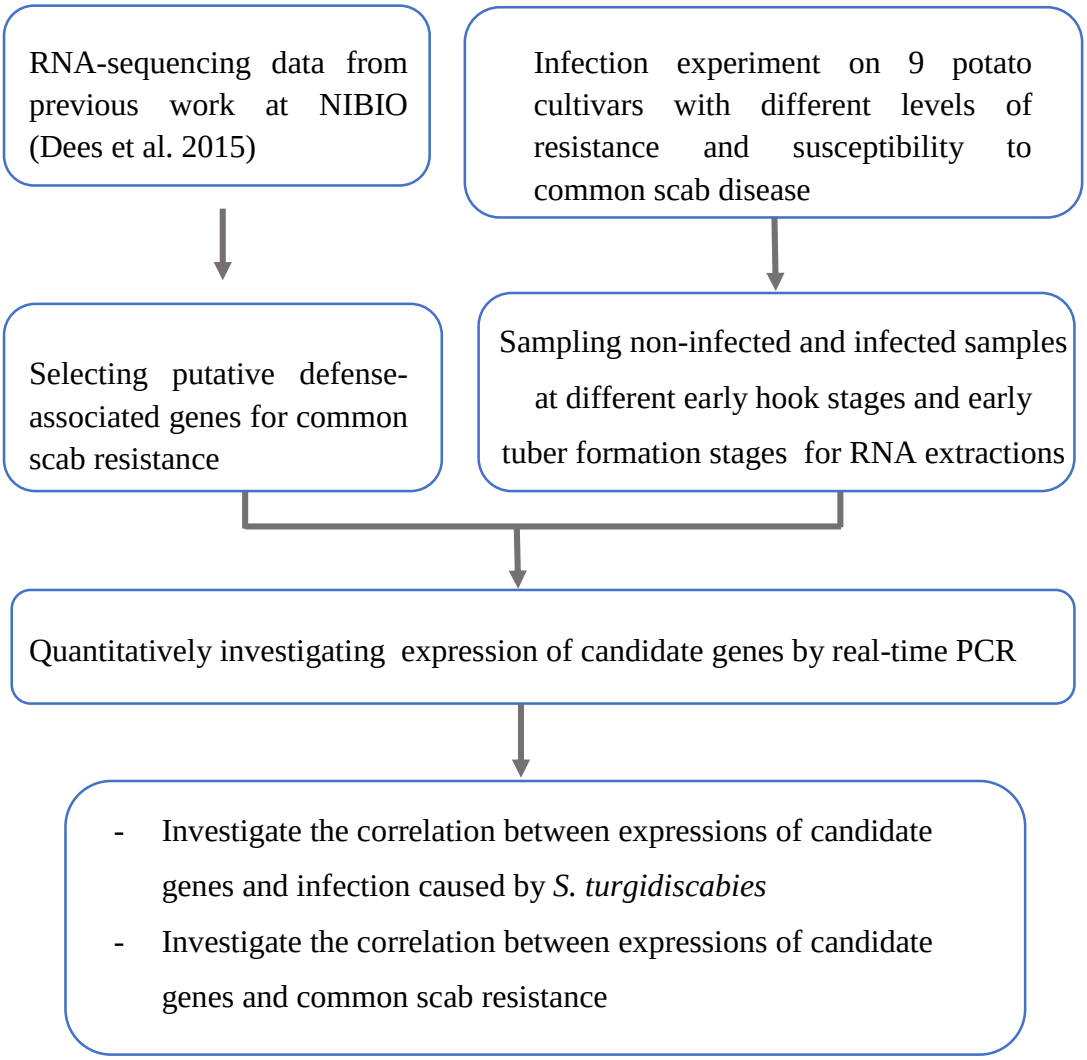
samples were achieved from the same infection experiment which was infected with the same *S. turgidiscabies* isolates used in RNA-sequencing samples.

1.10 Hypothesis

It was hypothesized that a number of candidate genes, uniquely expressed in resistant cultivar Beate, are associated with common scab resistance in potato, and therefore, they were hypothesized to perform similar expression patterns in other resistant cultivars.

1.11 Research strategies

The overview of research strategies carried out in this thesis is presented as below



2. MATERIALS

2.1 Facilities

- *S. turgidiscabies* cultures and pathogenicity assay on radish were carried out at laboratory V205, NIBIO, Norway.
- Infection experiment on potatoes was carried out at the Greenhouse of Norwegian University of Life Sciences, Norway.
- All molecular experiments were carried out at laboratory M108, NIBIO, Norway.

2.2 Laboratory equipment

Laboratory equipment	Supplier
Agilent 2100 Bioanalyzer	LabChip
Centrifuge	
- Table centrifuge	Thermo Scientic
- Cooling centrifuge	Eppendorf Centrifuge 5810R
Centrifuge tube	Nagene
Electrophoresis electricity supplier	
- PowerPac 300	Bio-Rad
Electrophoresis equipment	
- Agarose gel	Bio-Rad
Eppendorf tubes, 1.5ml standard	Axygen
Falcon tubes	Cellstar®TUBES
Freezer	Sanyo
Incubator	Termaks
Laminar hood	Thermo, KS12
Nanodrop spectrophotometer	Thermo Fisher Scientific
PCR plates, 96-well	Bio-Rad
Photo equipment	Bio-Rad
PCR machine	
- PTC-100 Programmable Thermal Controller	Bio-Rad
- Real-time PCR	Bio-Rad
Petri-dishes (d=9cm and d=15cm)	Sarstedt
pH meter 520 A	InoLab
5-litre pot	LOG AS, Oslo, Norway
Tips	Biotix
Horizontal vortexer	IKA Works

2.3 Chemicals

Chemical	Supplier
2-β-mercaptoethanol, C_2H_6OS	Sigma
Acetic acid, $C_2H_4O_2$	Sigma
Agar	Saveen
Agra-perlite	Pull Rhenen
Agarose	FCM Bioproducts
Bromphenol blue, $C_{19}H_{10}Br_4O_5S$	Sigma
Calsium chloride, $CaCl_2$	Merck
Dextrose	Difco
EDTA, $C_{10}H_{16}N_2O_8$	Sigma
Ethanol, C_2H_5OH	AntiBac
Ethidium bromide, EtBr	VWR
Oatmeal	Difco
Peat and clay	Tjerbo Torvfabrikk
Sodium hypochlorite	Merck
Sucrose, $C_{12}H_{22}O_{11}$	Duchefa
Tris-base, $C_4H_{11}NO_3$	Sigma
Tris-HCl, C4H11NO3xHCl	Sigma
Yeast extract	Difco

2.4 Potato cultivars

Seed tubers of 9 tetraploid potato cultivars were obtained from Graminor AS. Resistance score of each cultivar is presented in table 1.

Table 1: Potato cultivars by name and resistant score (Møllerhagen 2014)

Cultivars	Resistant scores
Beate	8
Mozart	8
Tivoli	7
Folva	6
Saturna	6
Pimpernel	4
DS x As	Not given
Gullauge	Not given
Hårek	Not given

2.5 Radish seeds

Organic radish seeds cultivar Rudolf (NORGRO).

2.6 Pathogens

Three thaxtomin A-producing isolates of *S. turgidiscabies* isolated by Dees et al. (2012), Bioforsk-08-45-02-3, Bioforsk-09-176-3-3 and Bioforsk-09-22-1-3, are from NIBIO.

2.7 Kits

Kits	Suppliers
Spectrum™ Plant total RNA kit - Lysis solution - Binding solution - Wash solution 1 - Wash solution 2 - Elution solution	Sigma-Aldrich, St. Louis, MO, USA
On-column DNase kit - Dnase digestion - Dnase 1	Sigma-Aldrich, St. Louis, MO, USA
Agilent RNA 6000 Nano kit - RNA gel matrix - RNA dye concentrate - Marker - Ladder	Agilent Technology
iScript™ Advanced cDNA synthesis kit for RT-qPCR - 5x iScript advanced reaction mix with dNTPs, oligo (dT) and random primers - iScript advanced reverse transcriptase, Rnase H+ MMLV reverse transcriptase and Rnase inhibitor - Nuclease free H_2O	Bio-Rad, CA, USA
Dneasy Plant Mini kit (250) - AP_1 lysis buffer - Rnase A - P_3 neutralization buffer - AW1 wash solution 1 - AW2 wash solution 2 - AE elution buffer	QIAGEN, Germany
SYBR Green	Bio-Rad

2.8 Media and agars

YME (Yeast Malt Extract Agar) medium	
Medium	4g yeast extract 10g malt extract 4g dextrose 1 liter dH_2O
pH	7.0 – 7.2
Agar	20g (2% w/v)

OMA (Oatmeal agar) medium	
Medium	72.5g Difco™ Oatmeal (containing 60g goat milk and 12.5g agar) 1 liter dH_2O

Medium was autoclaved at 121ºC for 15 minutes, cooled to about 55ºC and distributed 40ml into each sterile petri plate.

2.9 Growth-medium for potato plants

Mixture of P-soil 50:50 (v:v)	Agra-perlite (Pull Rhenen) Peat and clay (Tjerbo Torvfabrikk)
Pot	5-liter plastic pots (LOG AS)

2.10 Primers

Primers used are listed in Table 2. They were custom-made by Invitrogen

Table 2: Primers by name and sequence. These primers are for quantifying candidate gene expression by real-time PCR.

Potato gene name	NCBI NR-database	Forward primers		Reverse primers	
		Name	Sequence (5' to 3')	Name	Sequence (5' to 3')
PGSC0003DMG400040744	Late blight resistance	S.tub.62848.3f	AAATTTACGCTAGGGCTTG	S.tub.62848.3r	TCTCCTCTTTTCTCCTTTCTC
PGSC0003DMG400017087	Late blight resistance homologue r1a-10	S.tub.29871.f	TTGGGAAGCTAGAAGTGA	S.tub.29871.r	GGCGTTTTAGATTAGGGA
PGSC0003DMG400007385	Disease resistance protein at427190	S.tub.13075.f	GGAGAAAGTGATAGGTGG	S.tub.13075.r	ATGTGGTAGACGTTGAAG
PGSC0003DMG400010612	Disease resistance protein rpp13	S.tub.18753.f	CTGGCATCTTCACACAACT	S.tub.18753.r	CTCCCGTTGGCTTTGATT
PGSC0003DMG400013405	Myb-related protein myb4	S.tub.23726.f	GATGAGAGTTTTTGGACAGA	S.tub.23726.r	CCATCCGTAGTAGTAACA
PGSC0003DMG400000655	Serine-threonine-protein phosphatase 7	S.tub.01295.f	AGCGTCCTCAACAACAAAA	S.tub.01295.r	CTCTCGCTCTATCCACCA
PGSC0003DMG400015425	Sre 1a protein	S.tub.27024.f	TTTTTGTGTTTGGAGGGG	S.tub.27024.r	GAAAACAGAGAAGGGAGG
PGSC0003DMG400014859	Txr1 protein	S.tub.26162.f	TTGGCACGGTCTTTTGTT	S.tub.26162.r	CTGCATCAGTCATGGTTT
PGSC0003DMG400025578	Txr1 protein	S.tub.44341.f	TCGCCAAGCTCTCACAAA	S.tub.44341.r	TAGCCTCCACCTCTGTCAT
PGSC0003DMG402017989	Cyclic nucleotide-gated ion channel 1-like protein (CNG)	S.tub.31369.f2	TATCTCCCACATTACCTC	S.tub.31369.r	TGGTTCGGATGGTTTATG
PGSC0003DMG400022929	Aminotransferase ALD1	S.tub.39762.f1	TCGAGTTTATGGAGGTAG	S.tub.39762.r1	TGGAACCTGGGAAATGAA
PGSC0003DMG401008875	ATP binding cassette transporter B family (ABCB)	S.tub.15593.f2	GAGCTGTTGGTAAGACGA	S.tub.15593.r2	AAAGAGAGGAGCGAGGAA
PGSC0003DMG401020044	Auxin binding protein 1 (ABP1)	S.tub.34752.f2	TGAAAGAGATAGAGGTGTGG	S.tub.34752.r	AGGAGCAAGATAGAGAGT
PGSC0003DMG400003985	Actin	Potato.Actin.F	CCTCAGGGTTCAAGAAAAT	Potato.Actin.R	CTTTAGTAGTTGTGCCTGT
PGSC0003DMG400023270	Elongation factor 1-α	S.tub.ef1a.3f	TGGCGAGCATGATTTGGA	S.tub.ef1a.3r	CGCAACACCAAAAGCAAATA
PGSC0003DMG400044276	18SrRNA	S.tub.18SrR.f	CTGCCCTTTGTACACACC	S.tub.18rR.r	ACGACTTCTCCTTCCTCT

- NCBI NR-database, National Center for Biotechnology Information non-redundant database.

3. METHODS

3.1 Infection experiment on radish seeds

As radish is in the host range of pathogenic streptomycetes, infection experiment on radish is commonly used as a quick and low-cost method to confirm the production thaxtomin A. In case a *Streptomyces* isolate might be inactivated during a long storage at -80^{o}C, infection experiment on radish is also used to test for its activity. Prior to the infection experiment on potato plants in the greenhouse, *S. turgidiscabies* isolates were tested on radish for their ability to produce thaxtomin A. However, even when isolates are active in radish experiment, they might lose their activities when they grow in the soils. For the backup, three *S. turgidiscabies* isolates: Bioforsk-08-45-02-3, Bioforsk-09-176-3-3, and Bioforsk-09-22-1-3, were tested on radish for later use in infection experiment on potato plants.

3.1.1 Cultures of *Streptomyces turgidiscabies*

To produce mycelia and spores, three *S. turgidiscabies* isolates were separately cultured on YME solid agar plates, at 28^{o}C in dark for 4 days. All mycelia and spores from each isolate obtained from YME-cultures were separately collected. The production of thaxtomin A by virulent streptomycetes is only induced in living host tissues or media containing oat grains or oat bran. To induce production of thaxtomin A by three investigated *S. turgidiscabies* isolates, their mycelia and spores harvested from YME-cultures were separately transferred onto 3 plates of solid agar containing OMA-medium for each isolate. Then, 9 OMA-plates with 3 isolates were cultured at 28^{o}C in dark for 8 days.

3.1.2 Radish seed sterilization

Radish seeds were sterilized in ethanol (70%) for 30 seconds and sodium hypochlorite (0.5%) for 1 minute, then rinsed with sterilized distill water 3 times.

3.1.3 Experiment set-up

For infection, 14 sterilized radish seeds were placed deeply into each OMA-plate containing *S. turgidiscabies*, 3 plates for each isolate. Similarly, 14 sterilized radish seeds were placed deeply into OMA-plates without any *S. turgidiscabies* isolate as negative controls. To facilitate seed germination, a 15-cm petri plate was placed outside each cultured plate and

added with sterilized water for moisture (figure 6). All plates were cultured at room temperature for 7 days.

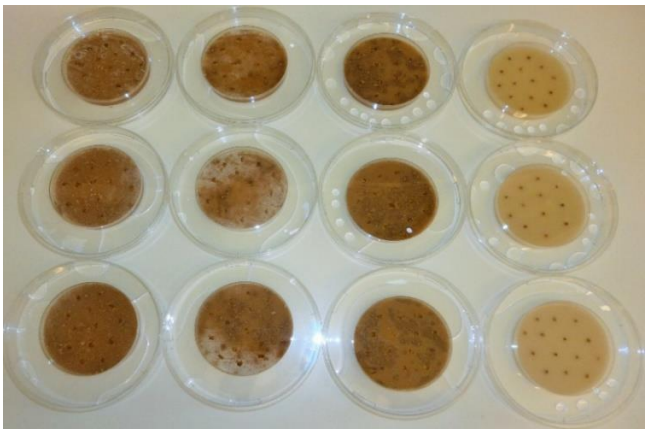


Figure 6: experiment setup for the infection experiment on radish. The first three rows from the left, with brown color in the medium and grey mycelium was observed on the surface, are OMA-plates containing *S. turgidiscabies* isolates. The last row on the right, with original color of OMA medium, is OMA-plates without *S. turgidiscabies* for negative control with. In each plate, 14 radish seed were sown.

3.2 Infection experiment on potato plants

3.2.1 Cultures of *S. turgidiscabies* isolates

The infection experiment on radish indicated that there *S. turgidiscabies* isolates produced thaxtomin A (discussed in section 5.1). Therefore, these three thaxtomin A-producing *S. turgidiscabies* isolates were used to cause common scab infection on potato plants grown in the soil.

Similar to experiment on radish, three *S. turgidiscabies* isolates were separately cultured on YME- medium solid agar plates, at 28^{o}C in dark for 4 days.

Figure 7: *S. turgidiscabies* after 8 days on YME-plate with grey mycelium.

All mycelia and spores of 3 isolates were harvested and thoroughly mixed together in 35ml sterilized distilled water. Then, 20µl of suspension were evenly spread out on the surface of

each YME-plate. All plates were placed upside down in an incubator, at 28°C, for 8 days. After 72 hours and before inoculating potato plants, all cultured plates were observed for grey and dry-looked mycelium formed on the surface to ensure no contamination (figure 7).

3.2.2 Inoculation of growth-medium and plant infection

To mix Agra-perlite with peat-soil and to homogenize *S. turgidiscabies* cultures in growth-medium, a concrete mixer was used. To avoid contamination, growth-medium for negative control was prepared prior to growth-medium for infection.

Procedure:

1. 10 liters of Agra-perlite were mixed with 10 liters of peat-soil by the concrete mixer.
2. Another 10 liters of Agra-perlite and 10 liters of peat-soil were added and thoroughly mixed.
3. Suspension of 16 plates of YME-medium (for negative control) or slurry of 16 plates *S. turgidiscabies* solid cultures (for infection) was finally added and thoroughly mixed.
4. The final mixture was distributed into 5-litre pots. On average, each pot contained 2 YME-plates for negative control or 2 *S. turgidiscabies* solid cultured plates for infection.

In each pot, one sprouted potato seed tuber was placed as deep as its size.

3.2.3 Experiment set-up

The mixture of 3 *S. turgidiscabies* isolates was used to inoculate 9 potato cultivars with various resistant scores (table 1). YME-medium solid agar, without *S. turgidiscabies*, was used as negative control. For each cultivar, 6 sprouted seed tubers were grown in 6 pots without *S. turgidiscabies* isolate for negative control and 6 sprouted seed tubers were grown in 6 pots inoculated with *S. turgidiscabies* for infection.

Pots of infected plants, labelled from 1 to 54 for 9 cultivars, were placed separately in 1 table in the greenhouse. Pots of non-infected plants, labelled from 55 to 108 for 9 cultivars, were placed separately in another table in the same greenhouse compartment. All pots were grown under the same condition, at 18°C under natural light for 4 weeks. In the first week, pots

were manually watered to keep moisture on the surface and dryness in the lower part to promote *S. turgidiscabies* growth. From second week onwards, an automated drip-irrigation system was mounted to supply water and fertilizer individually to each pot (figure 8).

Figure 8: Experiment set-up for infection experiment on potato cultivars. On the left is the table for negative control plants. On the right is the table for infected plants. Both table shared the same drip irrigation system.

3.2.4 Sampling

Sampling and symptom scoring were carried out after 4 weeks. Stolons and tubers were harvested and sorted out by 5 different growing stages as presented in figure 9.

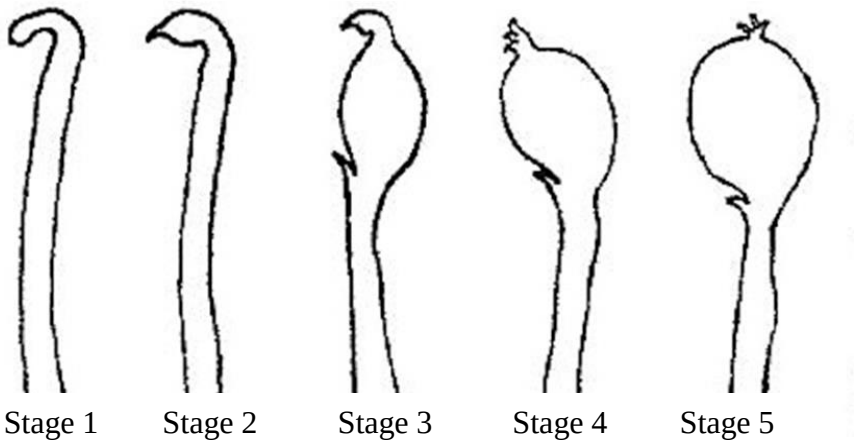


Figure 9: Descriptions of samples at 5 tuber developing stages. Stage 1 is early hook stage. Stage 2 is hook stage. Stage 3 is early tuber formation. Stage 4 and 5 are tuber formation. (Picture is taken from Nilsson et al. 2012).

All samples were snap-frozen in liquid nitrogen and stored at -80^{o}C until RNA extracted.

3.2.5 Symptom scores

To score symptom in each cultivar, surface area with lesion in each sample (hook or tuber) were measured. Symptom score for each cultivar was calculated based on the mean value of all areas performing symptoms in all collected samples in that cultivar.

3.3 RNA sample preparation and cDNA synthesis

RNA molecules play an important role in transferring encoded information in the DNA genome to many different forms of proteins. Therefore, RNA extracted from cells are used to measure cellular activities by gene expression measurement techniques.

Based on the numbers of samples obtained from infection experiment on 9 potato cultivars, only 6 cultivars had sufficient samples for RNA extractions, which are cultivar Beate, cultivar DSxAs, cultivar Gullauge, cultivar Pimpernel, cultivar Saturna and cultivar Tivoli.

3.3.1 RNA extraction

For gene expression analysis, RNA samples will be reverse transcribed and the resulting cDNA will be used as templates for expression analysis by real-time PCR.

As RNA is easily degraded by RNases found in the air and on every surface, including human skin. Therefore, samples were handled with precaution. All surfaces (bench, pipettes, and gloves) were washed with RNase Zap before each experiment. Tubes were closed as much as possible. Nuclease-free filter tips and nuclease-free tubes were used throughout experiments. All solutions were RNase free.

RNA extraction procedure was carried out in the fume hood due to the use of 2-β-mercaptoethanol.

Materials

Liquid nitrogen

Spectrum^{TM} Plant total RNA kit (Sigma-Aldrich, St. Louis, MO, USA)

- Lysis solution
- Binding solution
- Wash solution 1
- Wash solution 2
- Elution solution

On-column DNase kit

- DNase digestion
- DNase 1

Procedure:

1. Potato stolon or young tubers were ground in a sterilized mortar containing liquid nitrogen. 50mg of tissue powder were transferred into a 2-ml micro-centrifuge tube and kept in liquid nitrogen until processed.
2. For lysis step, lysis mixture was made including of 500µl of lysis solution and 5µl of 2-β-mercaptoethanol. Then, 500µl of lysis mixture were added into tissue powder and immediately vortexed vigorously for 30 seconds. Sample was then incubated at room temperature for 5 minutes.
3. To pellet cellular debris, sample was centrifuged at 14,000g for 3 minutes.
4. For lysate filter, the supernatant was collected into a filtration column placed in a 2-ml collection tube and centrifuged at 14,000g for 1 minute to remove residual debris. The filtration column was then discarded.
5. To bind RNA to column, 500µl of binding solution were added into flow-through lysate and mixed thoroughly by pipetting. 500µl of binding mixture were transferred into binding column placed in a 2-ml collection tube and centrifuged at 14,000g for 1 minute. The flow-through was discarded and these steps were repeated with the remaining binding mixture.
6. 300µl of wash solution 1 was added into binding column and centrifuged at 14,000g for 1 minute.
7. To remove genomic DNA, on-column DNase kit was used. 80µl of digestion solution, including 70µl of DNase digestion and 10µl of DNase 1, was added directly

onto the center of the filter, inside the binding column. Sample was incubated at room temperature for 15 minutes.

8. To remove digested DNA, 500µl of wash solution 1 was added into binding column and centrifuged at 14,000g for 1 minute. The flow-through was discarded and 500µl of diluted wash solution 2 were added into the column and centrifuged at 14,000g for 30 seconds. For the last wash, another 500µl of diluted wash solution 2 were added and centrifuge step was repeated at 14,000g for 30 seconds.
9. The column was dried out by centrifuging at 14,000g for 1 minute.
10. To elute the RNA, the column was transferred into a new collection tube and 50µl of elution solution were added directly onto the center on to the binding matrix inside the column for 1 minute. The tube was then centrifuged at 14,000g for 1 minute.
11. Eluted RNA in the collection tube was collected and returned to the column for second elution by centrifuging at 14,000g for 1 minute.
12. RNA was stored at -80^{o}C.

3.3.2 Detecting genomic DNA contamination by real-time PCR

Residual genomic DNA will be a source of false positives in subsequent expression analysis by real-time PCR. Therefore, it is very important that all RNA samples not contain residual genomic DNA.

Real-time PCR was used to test for purification of extracted RNA samples. Primers Potato.Actin.F and Potato.Actin.R of *PGSC0003DMG400003985* gene (table 2), were used to detect residual of genomic DNA in RNA samples. Samples with Cq mean value of 0,00 were considered genomic DNA free.

Materials:

- Power SYBR-green master mix
- Actin forward primers, 10pmol/µl
- Actin reverse primers, 10pmol/µl
- RNA free, DNA free H_2O

Procedure:

1. The reaction prepared for real-time PCR detecting gDNA is in table 3.

Table 3: master mix preparation for real-time PCR detecting gDNA

Reagents	Volume
Power SYBR-green master mix	10 µl
Actin forward primer	1.6 µl
Actin reverse primer	1.6 µl
RNA free, DNA free H_2O	5.8 µl
Total volume	19 µl

2. 19µl of master mix were added into each well of 96-well realtime PCR plate.
3. 1µl of RNA sample was added. Each sample was duplicated.
4. Potato gDNA was used for positive control.
5. RNA free, DNA free H_2O was used for negative control.

The reaction was initiated by 3 minutes incubation at 98^{o}C, and followed by 40 cycles of: 98^{o}C for 10 seconds to allow the separation of the nucleic acid double chain; and 60^{o}C for 30 seconds to allow the binding of the primers with the DNA template. The fluorescence is measured during short temperature phase, lasting only 5 seconds in each cycle, with a temperature between 65^{o}C to 95^{o}C.

3.3.3 Valuation of RNA integrity, quality and quantity by Nano Bioanalyzer

RNA is rapidly digested in the presence of ubiquitous RNase enzymes, resulting in shorter RNA fragments in the samples which potentially compromise results of gene expression study. To evaluate the RNA integrity and degradation degree, Nano analysis – Agilent 2100 Bioanalyzer was used. The bioanalyzer is an automated bio-analytical device based on microfluidics chips, voltage-induced size separation in gel filled channels and laser-induced fluorescence detection. Twelve samples can be sequentially processed in each micro-fabricated chips. RNA molecules are stained in intercalating dye, then separated in the channels of the chip according to its molecular weight and subsequently indicated by laser-induced fluorescence detection. Data are automatically archived and presented as electropherogram and gel-like images by 2100 Expert Agilent software. In which, RNA is considered of high quality if the ratio of 28S:18S bands is above 2.0; and the amount of

measured fluorescence indicates the concentration of RNA sample. RNA Integrity Number (RIN) is calculated by a software algorithm showing ten typical integrity categories ranging from 10 for intact RNA to 1 for totally degraded RNA (www.genomics.agilent.com).

Materials:

Agilent RNA 6000 Nano kit

- RNA gel matrix
- RNA dye concentrate
- RNA marker
- Ladder

Procedure:

1. To prepare gel mix, 550µl of RNA gel matrix were added into a spin filter, then centrifuged at 13,000g for 10 minutes at room temperature.
2. To prepare gel-dye mix, the RNA dye concentrate was equilibrated by leaving at room temperature for 30 minutes, with aluminum foil covered; then, vortexed for 10 seconds and spin down. 1µl of equilibrated RNA dye concentrate was added into 65µl gel mix and thoroughly mixed. The solution was centrifuged at 13000g for 10 minutes at room temperature.
3. To load the gel-dye mix, a new RNA chip was placed on the chip priming station with the plunger positioned at 1ml; 9µl of gel-dye mix was added into the well marked **G**; the chip priming station was then closed; the plunger was pressed until it was held by the clip for 30 seconds; the clip was then released.
4. After 5 seconds, the plunger was slowly pulled back to 1ml position. Another 9µl of gel-dye mix was added into the wells marked G
5. To load the marker, 5µl of RNA marker were added into all 12 sample wells and in well marked ladder.
6. 1µl of ladder was added into well marked ladder.
7. 1µl of each RNA sample was added into 12 sample wells
8. The chip was placed in the horizontal IKA vortexer and vortexed for 1 minute at 2480rpm.

9. The chip was run in the Agilent 2100 Bioanalyzer.
10. The concentrations of RNA samples and RIN values were indicated by 2100 expert Plant RNA Nano program.

Concentrations and RIN values of all RNA samples are presented in Appendix A.

3.3.4 The synthesis of cDNA

Reverse transcription is the process by which mRNA is copied into complementary DNA (cDNA). The mRNA in a cell is from protein-coding gene. Therefore, cDNA will represent the genes express in the cell from which the mRNA is extracted. An oligi (dT)-primer hybridizes to the poly(A) tail on mRNA, and enables the first strand of cDNA to be synthesized by reverse transcriptase enzyme.

Materials

iScript^{TM} Advanced cDNA synthesis kit for RT-qPCR

- 5x iScript advanced reaction mix with dNTPs, oligo (dT) and random primers
- iScript advanced reverse transcriptase, RNase H^+ MMLV reverse transcriptase and RNase inhibitor
- Nuclease free H_2O

Procedure

To synthesis cDNA with the same concentration in all samples, 3µg of each RNA sample were used in each reaction with total volume of 20µl. Reaction setup for a single cDNA synthesis is presented in table 4

Table 4: Reaction preparation for a single cDNA synthesis

Component	Volume and amount
5x iScript advanced reaction mix	4µl
iScript advanced reverse transcriptase	1µl
RNA template	3 µg (volume varies upon RNA concentration)
Nuclease-free water	volume varies upon RNA concentration
Total volume	20µl

All reactions were incubated in PCR machine at

- 42^{o}C for 30 minutes
- 85^{o}C for 5 minutes

Then, all cDNA samples were stored at -20^{o}C

3.4 Gene expression studies

3.4.1 Selection of candidate genes with potential involvement in enhanced resistance to common scab

RNA-sequencing is a useful tool for candidate gene selections because it reveals RNA presence and measures RNA levels at a sampling time. RNA-sequencing also can be used to compare transcript profiles between healthy cells and disease cells (Lesk 2012). In this thesis, RNA-sequencing data from previous work at NIBIO were first used to observe the expression of number of genes putatively involved in common scab disease resistance. The selection was based on the predicted function of a single gene and its different expression patterns in the resistant cultivar Beate and the susceptible cultivar Saturna.

The transcript profiles of candidate genes are presented in Appendix A.

3.4.1.1 Genes uniquely expressed in the resistant cultivar Beate in RNA-sequencing

As indicated by Dees et al. (2015), a number of genes encoding putative disease resistance proteins and putative proteins related to defense regulations were uniquely expressed in the resistant cultivar Beate when the host plant was subjected to *S. turgidiscabies*. Hence, these genes were hypothesized to be related to common scab resistance in potato.

Gene *PGSC0003DMG400040744*, with predicted function of late blight resistance protein, uniquely expressed in cultivar Beate (Dees et al. 2015).

Gene *PGSC0003DMG400017087*, with predicted function of late blight resistance protein homologue r1a-10, uniquely expressed in cultivar Beate (Dees et al. 2015).

Gene *PGSC0003DMG400007385*, with predicted function of disease resistant protein At4g27190 in *Arabidopsis thaliana* (Mayer et al. 1999), uniquely expressed in cultivar Beate (Dees et al. 2015).

Gene *PGSC0003DMG400010612*, with predicted function of disease resistant protein rpp13, was indicated as resistance protein to downy mildew in *A. thaliana* without salicylic accumulation (Bittner-Eddy & Beynon 2001) and uniquely expressed in cultivar Beate (Dees et al. 2015).

Gene *PGSC0003DMG400013405,* with predicted function of myb-related protein myb4, was indicated to regulate plant defense upon pathogen attack in *A. thaliana* by Van Verk et al. (2009) and uniquely expressed in cultivar Beate (Dees et al. 2015).

Gene *PGSC0003DMG400000655*, with predicted function of serine threonine-protein phosphatase-7, was indicated involving in cross regulation of various pathways response to light and stress signaling (Andreeva & Kutuzov 2009) and uniquely expressed in cultivar Beate (Dees et al. 2015).

Gene *PGSC0003DMG400015425,* with predicted function of Sre1a protein, was indicated to be induced during systemic acquired resistance in potato (Wegener et al. 1996) and one of its isoform uniquely expressed in cultivar Beate (Dees et al. 2015).

3.4.1.2 Genes putatively involved in thaxtomin A transport

Genes *PGSC0003DMG400014859* and *PGSC0003DMG400025578* have predicted function of txr1-like novel proteins participated in thaxtomin transport into plants cells. This function was characterized in *Arabidopsis* by Scheible et al. (2003). RNA-sequencing data shown that these genes similarly expressed in cultivar Beate and cultivar Saturna (appendix A).

Gene *PGSC0003DMG402017989* has predicted function of cyclic nucleotide-gated ion channel 1-like protein (CNGs). It was discussed that CNGs might be candidates promoting thaxtomin A interaction with the cellular memebrane in *Arabidopsis* (Tegg et al. 2005). RNA-sequencing data showed that this gene was up-regulated at early hook stage in infected samples of susceptible cultivar Saturna (appendix A).

3.4.1.3 Gene putatively involved in the generation of amino acid homeostasis and defense response

Gene *PGSC0003DMG400022929,* with predicted function of aminotransferase ALD1, was suggested to be involved in the generation of amino acid homeostasis in the cell and in

generation of amino acid-derived defense signal (Kachroo & Robin 2013; Song et al. 2004; Zeier 2013). This gene uniquely expressed at early hook stage of susceptible cultivar Saturna (appendix A).

3.4.1.4 Genes putatively involved in auxin signaling

Gene *PGSC0003DMG401008875,* with predicted function of ATP binding cassette transporter B family (ABCB), was suggested to be involved in auxin transport in *Arabidopsis* (Lin & Wang 2005; Taiz & Zeiger 2010). RNA-sequencing data showed higher expression of this gene in resistant cultivar Beate than that in susceptible cultivar Saturna (appendix A). This gene was selected to investigate a putative correlation between auxin level in the cell and common scab resistance as presented in section 1.7.3.2, (figure 4).

Gene *PGSC0003DMG401020044,* with predicted function of auxin binding protein 1 (ABP1), has been suggested to be a key component in auxin signaling as it binds auxin to auxin receptor (Napier et al. 2002; Taiz & Zeiger 2010; Tromas et al. 2009) and activates the transcription of auxin- inducible gene *GH3* (Leyser 2006) (presented in section 1.7.3.2, figure 4). This gene was selected to investigate a putative correlation between auxin signaling and common scab resistance.

3.4.1.5 Reference genes for real-time PCR normalization

Comparing expression of gene in different biological samples requires normalization to compensate for the heterogeneity. Normalization to internal reference gene is currently most popular. Finding internal reference genes which are stably expressed during treatments or disease development is very important. Several studies on reference gene for real-time PCR normalization in potato have been reported. Elongation factor 1-α (ef1 α), actin and 18S rRNA were indicated most stably-expressed in potato tuber tissues during biotic and abiotic stress (Nicot et al. 2005). In another study, six reference genes including actin, APRT, 18S rRNA, ef1α, β-tubulin and ribosomal protein L2, were evaluated as reference genes in potato tuber tissues of five cultivars during cold stress (Lopez-Pardo et al. 2013). The study indicated that any of these six genes could be used for reference gene, and the most stably expressed gene is ef1α. In this thesis, three internal reference genes were selected:

PGSC0003DMG400003985 putatively coding actin, *PGSC0003DMG400023270* putatively coding elongation factor 1-α and *PGSC0003DMG400044276* putatively coding 18S rRNA.

3.4.2 Sequence identification

Potato coding sequence ID (CDS ID) of candidate genes were obtained from RNA-sequencing data. The link between gene ID, transcript ID, CDS ID, and putative function is obtained from PGSC_DM_v3.4_g2t2c2p2func_nonredundant.txt.zip (Potato Genomics Resource page: http://solanaceae.plantbiology.msu.edu/index.shtml).

From CDS ID, the coding sequence of candidate genes were obtained from PGSC_DM_v3.4_cds.fasta.zip (Potato Genomics Resource page).

The putative functions of the genes were checked by BLASTx against non-redundant protein sequences in all species, using http://blast.ncbi.nlm.nih.gov/Blast.cgi.

3.4.3 Primer designs

Primers were designed based on the last 300-base-pair region of the gene, using CLC Work Bench Version 6.9.1. These primer pairs were aimed to amplify fragments less than 150 base pairs. To make sure each primer pair will amplify only fragments from targeted gene, the programme http://www.ncbi.nlm.nih.gov/tools/primer-blast/ was used to test for primer specificity. Sequences of primers used in this thesis were presented in section 2.10 (table 2).

3.4.4 Real-time PCR amplification efficiency analysis

Information about the performance of real-time PCR reaction and reaction parameters including the correlation coefficient value and slope value can be obtained from a standard curve. The ideal amplification efficiency (E) of a real-time PCR reaction is 100% when the template is doubled after each exponential amplification cycle. The actual efficiency indicates information about the reaction such as the length of amplicons, GC contents of amplicons as well as the dynamic of the reaction. For instance, non-optimal reagent concentrations or enzyme quality can lead to efficiencies below 90%. Efficiencies can be greater than 110% in case of PCR inhibitors in one or more of the reagents. A successful reaction should have an efficiency between 90% and 110%, and a slope of between -3.58 and -3.10. Efficiency of 100% corresponds to a slope of -3.32. A PCR efficiency can be

determined by the following equation: Efficiency = $10^{(-1/slope)}$ -1. The correlation coefficient (R^2) indicates how well the data fit the standard curve. The ideal R^2 value is 1, although 0.999 is generally the maximum value.

To establish a standard curve, a dilution series of known cDNA template concentrations was set up. The log of each known concentration in the dilution series was plotted against the Ct value for that concentration.

For candidate genes selected based on their higher expression in cultivar Beate, cDNA infected stage-1 sample of cultivar Beate (sample ID: 5s1 in appendix B) was used as templates with tenfold serial dilutions for primer efficiency tests. Likewise, for candidate genes elected based on their higher expression in cultivar Saturna, cDNA infected stage-1 sample of cultivar Saturna (sample ID: 48.s1 in appendix B) was used.

Procedure

1. Five tenfold dilutions of cDNA, with concentrations in the range of 0.03ng to 300ng, were prepared as below

- 1:10 dilution (300ng): 180µl water were thoroughly mixed with 20µl cDNA sample.
- $1:10^2$ dilution (30ng): 180µl water were thoroughly mixed with 20µl 1:10 dilution.
- $1:10^3$ dilution (3ng): 180µl water were thoroughly mixed with 20µl $1:10^2$ dilution.
- $1:10^4$ dilution (0.3ng): 180µl water were thoroughly mixed with 20µl $1:10^3$ dilution.
- $1:10^5$ dilution (0.03ng): 180µl water were thoroughly mixed with 20µl $1:10^4$ dilution.

2. For each primer pair, these 5 dilutions were used as templates in real-time PCR and each dilution was duplicated. DNA-and-RNA-free-water was used for negative control.

3. Master mix preparation for each reaction is presented in table 5

Table 5: Master mix preparation for amplification efficiency test of each reaction

Reagents	Volume for 1 reaction
SYBR Green	10µl
Forward primer, 10pmol/µl	1µl
Reverse primer, 10pmol/µl	1µl
RNA&DNA free H_2O	6µl
Total volume	18µl

4. 18µl master mix were added into each well of real-time PCR plate.

5. 2µl of cDNA dilution was added. Each dilution was duplicated.

6. RNA free, DNA free H_2O was used for negative control.

7. Running information:

The reaction was initiated by 3 minutes incubation at 95ºC, and followed by 40 cycles of: 95ºC for 10 seconds to allow the separation of the nucleic acid double chain; and 55ºC for 30 seconds to allow the binding of the primers with the DNA template. The fluorescence is measured during short temperature phase, lasting only a 5 seconds in each cycle, with melt curve temperature between 65ºC to 95ºC.

8. Standard curve, slope and melting curve were created using CFX_Manager software (Bio-Rad). The specific primer pairs were expected to perform 1 pattern of melting curve. Information about reaction parameters including the correlation coefficient value, slope value and amplification efficiencies of all candidate genes were determined.

3.4.5 Real-time PCR for gene expression studies

A real-time PCR method is based on the detection and quantification of a fluorescent reporter such as SYBR® Green. The amount of fluorescence emission (ΔRn) is recorded at each cycle in a PCR reaction and monitored during the exponential phase. Thereby, the initial amount of the target template can be quantified based on the amount of PCR product.

The parameter Ct (threshold cycles) is the cycle number at which the fluorescence emission (ΔRn) exceeds the threshold and first be detected. The Ct value and amplification curves are obtained by plotting the fluorescence emission against the cycle numbers in the PCR reaction. The higher initial amount of DNA template, the sooner PCR product is detected in the PCR process, and thus the lower Ct value will be indicated.

After primer efficiency test, expression of internal reference genes for normalization were investigated in all cDNA samples of all cultivars at stage 1, stage 2, stage 3 and stage 4 for their stable expression in all tuber development stages.

Master mix preparation for each reaction is as presented in table 5 and 1:10-dilution (300ng) cDNA samples were used throughout gene expression assay.

Running information:

1: 98°C for 3:00

2: 98°C for 0:10

3: 60°C for 0:30

Plate read

4: GOTO 2, 39 more times

5: Melt curve 65°C to 95°C: Increment 0,5°C 0:05

Plate read

After real-time PCR amplification, a melt curve will be achieved, which indicates the change in relative fluorescence units (RFU) with time (T). At the melting temperature, when two strands of DNA are separated, the fluorescence is released and the RFU curve will peak. Then, RFU will rapidly decrease afterwards.

3.4.6 Gene expression analysis

Expression of 3 internal reference genes, putatively encode actin, 18SrRNA, and elongation factor 1α, were obtained by real-time PCR method. The Ct values indicated that expression of gene putatively encoding actin was most stable in all potato cultivars during the treatments (appendix D). The amplification efficiency analysis and dissociation curve analysis of this gene also indicate that primers are specific with high efficiency (presented in section4.3.1) Therefore, *PGSC0003DMG400003985* putatively coding actin was selected for normalizing gene expression analysis in this thesis (discussed in section 5.3).

CFX Manager™ software was used to evaluate relative differences in a target's concentration between investigating samples. Gene expression data can be viewed in several ways consisting of bar chart, clustergram, scatter plot, volcano plat and heat map. In this thesis, gene expression data were presented as target combination by bar chart.

Procedure

1. Quantitative PCR data of reference gene and investigating gene were added into gene study setup.

2. "Normalized expression ΔΔCq" was selected as analysis mode from the Mode dropdown menu to normalize the relative quantity of candidate gene to relative quantity of reference gene across samples.
3. "Relative to zero" was selected from the Graph Data dropdown menu to display graph data relative to zero.
4. *PGSC0003DMG400003985* putatively coding actin was selected as reference gene in "Experiment settings" with auto efficiency.
5. The bar chart performing normalized fold expression was displayed together with a spreadsheet performing expression values and expression standard errors.
6. Expression values and expression standard errors were exported to excel file.
7. Since 3 RNA samples were represented in each group of samples as presented in appendix B, mean value of 3 normalized fold expression from those 3 RNA samples was calculated to present as the final gene expression results (presented in sections 4.3.2, 4.3.3, 4.3.4, and 4.3.5).

3.5. Presence of candidate genes in the genome

Since there were several genes did not express in some cultivars (figure 25, 27, 31), the presences of these genes in the genomes needed to be confirmed by real-time PCR. Genomic DNA of each cultivar was used as template to detect the presence of non-expressed candidate gene in the genome by real-time PCR method using the same primers used for gene expression (table 2).

3.5.1 Genomic DNA extraction

Materials

A biological sample of each investigating potato cultivar, either stolon or tuber, was selected as a representative for that cultivar.

For each potato cultivar, 3 samples of any stage were collected and separately extracted DNA. The list of 18 samples for 6 cultivars is presented in appendix C.

DNeasy Plant Mini kit (250) was used.

- AP_1 lysis buffer

- RNase A
- P_3 neutralization buffer
- AW1 wash solution 1
- AW2 wash solution 2
- AE elution buffer

Procedure

1. Samples were ground in a sterilized mortar containing liquid nitrogen. 100mg of powder were transferred into a 2-ml micro-centrifuge tube.
2. For lysis step, 400µl AP1 lysis buffer and 4µl RNase A were added, then vortexed for 1 minute and incubated at 65^{o}C for 10 minutes. During the incubation, the tube was inverted 3 times.
3. 130µl neutralization buffer P3 was added, vortexed for 30 seconds and incubated on ice for 5 minutes.
4. The lysate was centrifuged at 14,000g for 5 minutes.
5. For lysate filter, 500µl of the lysate was pipetted into a QIA shredder pin column placed in a 2-ml collection tube and centrifuged at 14,000g for 2 minutes.
6. 460µl of flow-through was transferred into a new tube. 690µl of wash solution 1 buffer AW1 were added and thoroughly mixed by pipetting.
7. For DNA binding, 650µl of the mixture were transferred into a DNeasy mini spin column placed in a 2-ml collection tube, centrifuged at 8,000g for 1 minute. The flow-through was discarded and the rest of the mixture was transferred into DNeasy mini spin column to repeat centrifuge step.
8. The spin column was transferred into a new 2-ml collection tube, added with 500µl wash solution buffer 2 AW2 and centrifuged at 8,000g for 1 minute. Another 500µl wash solution buffer 2 AW2 were added and centrifuged at 14,000g for 2 minutes.
9. To elute the DNA, the column was transferred into a new 2-ml collection tube, added with 50µl elution buffer AE and incubated at room temperature for 5 minutes. The tube was centrifuged for 1 minute at 8,000g. This eluting step was repeated with another 50µl elution buffer AE added.

3.5.2 Visualization of DNA integrity - Agarose gel electrophoresis

The DNA integrity was visualized by agarose gel electrophoresis. To separate bands larger than 900 base pairs, agarose gels were made in a 1% (w/v) concentration. Ethidium bromide was added to the gels to visualize the bands. Different DNA ladders were used to determine the size and concentration of samples.

Materials:

Agarose	
TAE-buffer (Tris-acetate), 50x	242g Tris-base 57.1 ml acetic acid 100ml 0.5 M EDTA, pH 8.0 Distil water to 1 litre
Ethidium bromide, 10mg/ml	
Loading dye (10x)	40% (w/v) sucrose
Sterile water to 10ml	0.25% bromphenol blue

Procedure:

1. To prepare 1% gels, 0.5g agarose was added into 50ml 1xTAE buffer. The solution was heated in the microwave until the agarose was completely dissolved.
2. The solution was cooled down to 60^{o}C and 1 drop of ethidium bromide was added. The solution was mixed and poured into a molding tray.
3. The molding tray with solidified gel was placed into an electrophoresis chamber which was filled up with 1xTAE-buffer.
4. 1µl 10x loading dye was mixed with 1µl DNA sample and loaded onto the gel.
5. The gel was run at 80V for 45 minutes.
6. The bands were visualized by UV light.

3.5.3 Genomic DNA confirmation

Plant cytochrome oxidase (COX)-specific primers (CoxF and CoxR) and COX probes were designed to detect plant DNA. The amplification of cytochrome oxidase fragment confirms that DNA was successfully extracted from potato samples (Tomlinson et al. 2005).

Materials

Plant cytochrome oxidase kit (Bio-Rad)

- 2x Bio-Rad Advanced Universal probe Mastermix
- Forward primer, 10pmol/µl
- Reverse primer, 10pmol/µl
- Probe, 5pmol/µl

Procedure

1. The reaction prepared for each real-time PCR to detect the presence of plant cytochrome oxidase is presented in table 6.

2. 18µl of master mix were added into each well.

3. 2µl of DNA sample, 1:20 dilution was added. Each sample was duplicated.

4. DNA free, RNA free H_2O was used for negative control.

Table 6: reaction prepared for each real-time PCR to detect the presence of plant cytochrome oxidase

Reagents	Volume
2x Bio-Rad Advanced Universal probe Mastermix	10µl
Forward primer, 10pmol/µl	0,2µl
Reverse primer, 10pmol/µl	0,2µl
Probe, 5pmol/µl	0,1µl
DNA free, RNA free H_2O	7,5µl
Total volume	18µl

5. Running information:

1: 95,0ºC for 3:00

2: 95,0ºC for 0:05

3: 60,0ºC for 0:20

Plate read

4: GOTO 2, 44 more times

3.5.4 Assessment of the purity of DNA

Nanodrop spectrophotometer measures the UV absorbance at wavelength of 260nm for both DNA and RNA, and at wavelength of 280nm for protein. A ratio of absorbance at 260/280nm

indicates the purity of DNA and RNA. A ratio of about 1.8 is expected for pure DNA. The list of DNA samples and their concentrations, 260/280 ratio is presented in appendix C.

3.5.5 Presences of candidate genes in potato genomic DNA

The presences of candidate genes in genomes were confirmed by real-time PCR. In which, the same primer pairs of each gene were used to amplify fragments in DNA samples of each cultivar used as templates.

Master mix preparation for each reaction is presented in table 3 and 2µl of 1:20 dilution DNA samples were used as template in gene presence assay.

Running information: The reaction was initiated by 3 minutes incubation at 98^{o}C, and followed by 40 cycles of: 98^{o}C for 10 seconds to allow the separation of the nucleic acid double chain; and 60^{o}C for 30 seconds to allow the binding of the primers with the DNA template. The fluorescence is measured during short temperature phase, lasting only 5 seconds in each cycle, with a temperature between 65^{o}C to 95^{o}C.

The Ct values indicating the presences of the investigating genes are presented in appendix D.6

4. RESULTS

4.1 Infection experiment on radish seeds

In OMA-plates with *S. turgidiscabies* isolates, the medium turned brown and the surface was covered with grey mycelium, whereas, OMA-plates without *S. turgidiscabies* isolate still remained original color of OMA medium (figure 6). When radish seeds were sown on OMA-plates without *S. turgidiscabies* isolate, the original color of OMA medium still remained. While, OMA-plates with *S. turgidiscabies* isolates remained brown color in the medium (figure 10).

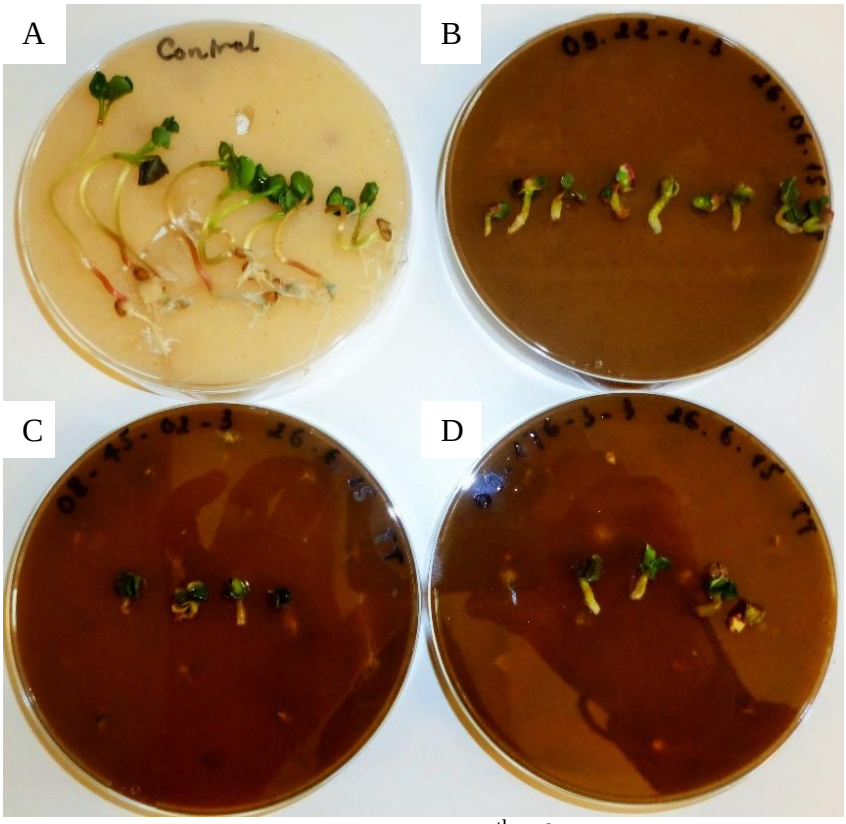


Figure 10: Radish seedlings observed on day 7th of the experiement. (**A)** On OMA-plate without *S. turgidiscabies* isolates for control, normal seedlings grown and medium remained original color of OMA medium. On OMA-plates with *S. turgidiscabies* isolates (**B)** 09-22-1-3, (**C**) 08-45-02-3, (**D**) 09-176-3-3, stunting seedling without roots, hypertrophy and necrosis on leaves occurred.

Differences in radish seedling growth on negative control plates and *S. turgidiscabies* plates were observed. On negative control plates, seeds normally germinated and elongated with green leaves, white and long roots. The longest seedlings were about 7cm, the shortest ones

were about 2cm (figure 10A), whereas, on plates with *S. turgidiscabies* isolates, stunting seedlings were observed with lengths ranging from 0.5cm to 1cm, with necrosis on the leaves, hypertrophy and without roots (figure 10B, C and D).

There were significant differences in seed germination rates. Without *S. turgidiscabies*, 71% and 83% of seeds germinated after 3 days and 7 days respectively. Whereas, the number of seeds germinated on plates with *S. turgidiscabies* isolates were much lower. The lowest percentage of germinated seeds was on plates infected with isolate 08-45-02-3, 29% after 3 days; no more seeds germinated afterwards and 5% of germinated seeds died after 7 days. On plates infected with isolate 09-176-3-3, 33% of seeds germinated after 3 days and no more seed germinated afterwards. On plates infected with isolate 09-22-1-3, 43% of seeds germinated after 3 days and few more germinated after 7 days (table 7).

Table 7: number and percentages of seeds geminated after 3 days and 7 days being sowed on each isolate

S. turgidiscabies isolates	Plates	After 3 days		After 7 days	
		Number of germinated seed	%	Number of germinated seed	%
08-45-02-3	1	3	29	3	24
	2	3		3	
	3	6		4	
09-176-3-3	1	4	33	4	33
	2	6		6	
	3	4		4	
09-22-1-3	1	4	43	6	52
	2	9		9	
	3	5		7	
Negative control	1	10	71	11	83
	2	10		11	
	3	10		13	

Note: 14 seeds in each plate.

4.2 Infection experiment on potato plants

All cultivars formed stolons (hooks) and some cultivars started forming tubers (figure 11). Only cultivar Beate did not perform any lesions in both non-infected and infected plants (figure 12).

Figure 11: Different stages of tuber formation in cultivar Beate, ranging from hook stage to tuber bulking stage.

Figure 12: Non common scab symptom stolons of cultivar Beate at different stages. (From left to right: stage-1, stage-2 and stage-3 samples) (Photo: Vinh Le).

Whereas, brown superficial corky lesions were observed on the surface of the hooks and tubers of all non-infected and infected plants of cultivar DSxAs, cultivar Folva, cultivar Gullauge, cultivar Harek, cultivar Pimpernel, cultivar Saturna and cultivar Tivoli (figure 13).

Figure 13: Brown superficial corky lesions on the surface of a stolon (Photo: Vinh Le).

Areas of lesions were measured for symptom scores. The percentages of lesion areas on tuber surface varied within cultivars (table 8). All stolons and tubers in cultivar Mozart cultivar got rotted.

Table 8: Percentages of lesion areas recorded in 9 investigating cultivars

Cultivars	Resistance scores by Møllerhagen (2014)	Percentages of lesion areas (%)	
		Non-inoculated plants	Inoculated plants
Beate	8	0	0
Mozart	8	Stolons and tubers rotted	
Tivoli	7	6	8
Folva	6	15	19
Saturna	6	10	14
Pimpernel	4	25	29
AD xAs	Not given	10	15
Gullauge	Not given	19	24
Hårek	Not given	4	9

4.3. Gene expression analysis

4.3.1 Expression of reference genes for normalization

Based on the Ct values, standard curves were created to calculate primer amplification efficiencies. Melting curve analyses were established to identify specific amplicons generated during PCR reactions and to ensure primers did not amplify unspecific fragments during 40 amplification cycles. In order to select the most reliable reference gene for normalization, melting curves obtained from quantitative expression of reference genes in samples at all stages of all investigated cultivars were analyzed.

4.3.1.1 Expression of *PGSC0003DMG400003985* gene, putatively encoding actin

The standard curve indicates that Ct values of duplicates of each concentration of cDNA input, ranging from 0.03ng to 300ng, were within the range of the standard curve (figure 14).

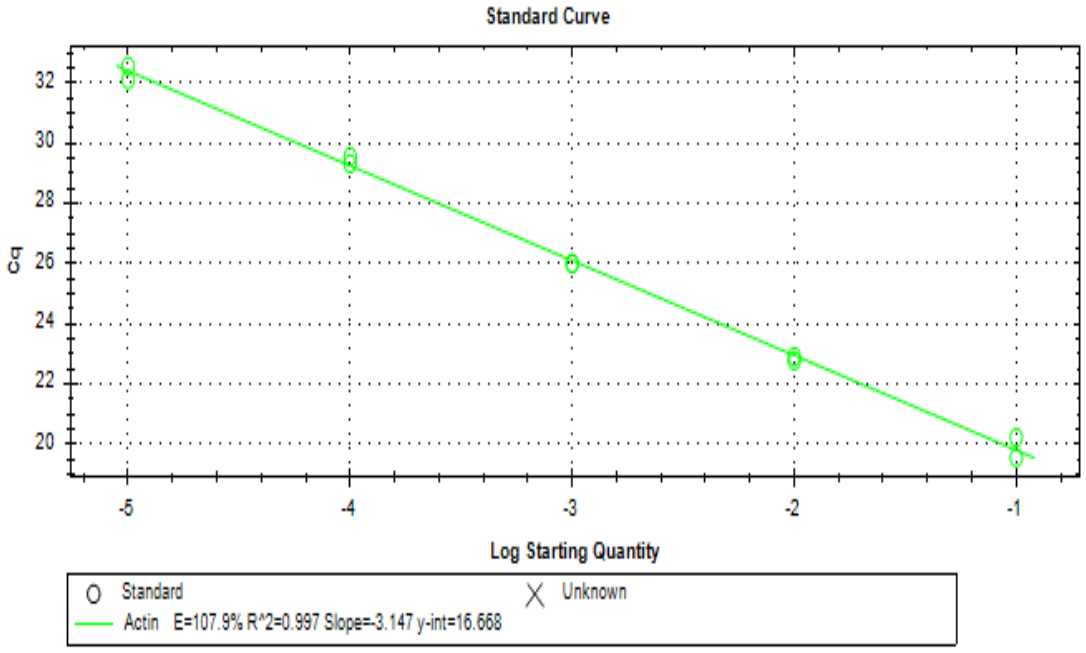


Figure 14: Regression line for quantity of primer amplification efficiencies analysis from quantitative expression of *PGSC0003DMG400003985* gene, putatively encoding actin, in stage-1 infected sample of cultivar Beate with template cDNA input in the range of 0.03ng to 300ng. Efficiency is calculated following equation E 107.9%.

The analysis indicates the correlation coefficient (R^2) of 0.997, the lope of -3,147 in the range of successful reactions (between -3.58 and -3.10) and the amplification efficiency of 107.9% in the range of successful reactions (between 90% and 110%).

The melt curve analysis of quantitative expression of the gene in samples of four stages in tuber development consistently show only 1 peak at 83ºC (figure 15A, B, C, and D).

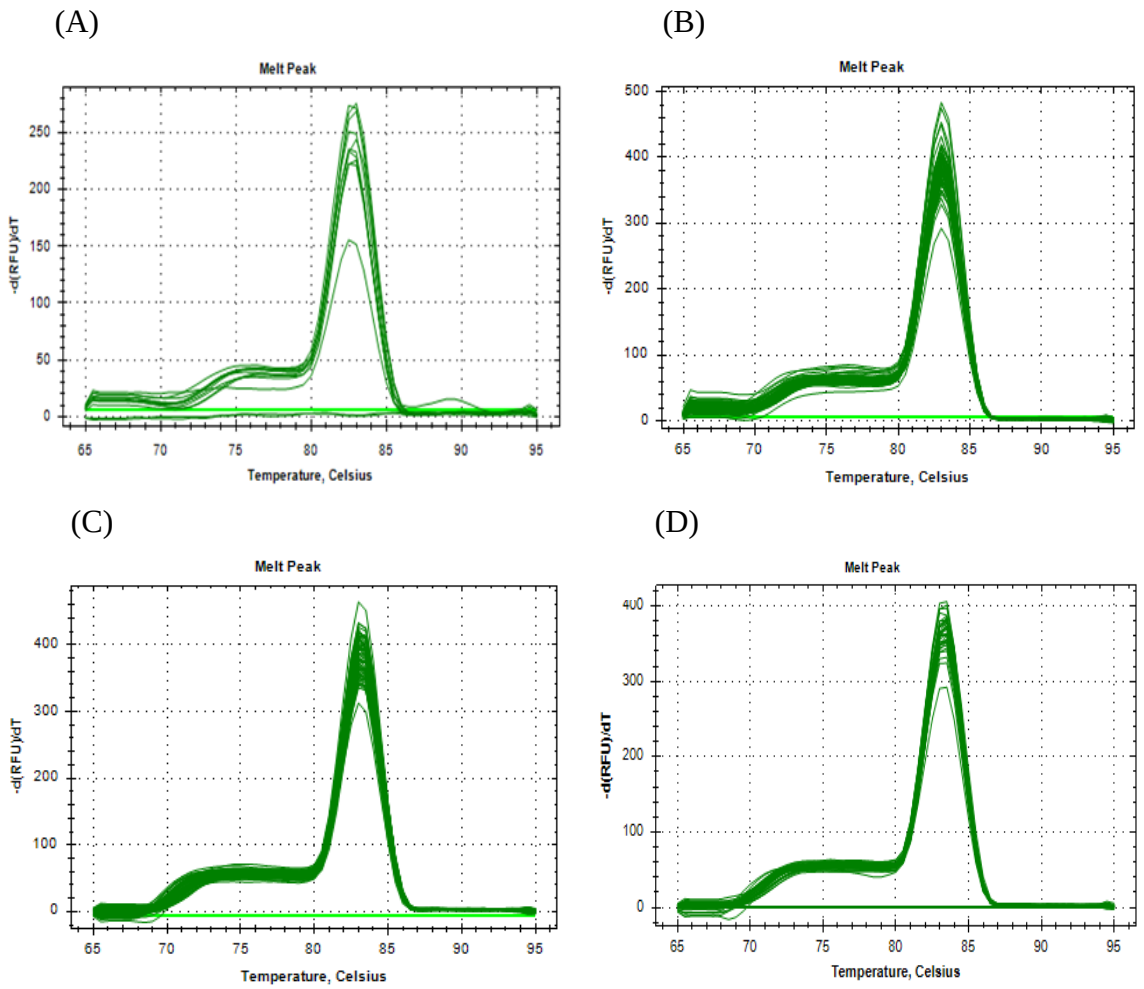


Figure 15: The dissociation (melting) curve analyses from quantitative expression of *PGSC0003DMG400003985* gene, putatively encoding actin, were conducted to identify amplicons produced during PCR. (**A**) Stage-1 infected sample of cultivar Beate with template cDNA input in the range of 0.03ng to 300ng. (**B**) Stage-1 infected and non-infected samples of 6 cultivars with template cDNA input of 300ng. (**C**) Stage-2 infected and non-infected samples of 6 cultivars with template cDNA input of 300ng. (**D**) Stage-3 and stage-4 infected samples of 3 cultivars with template cDNA input of 300ng. Figures A, B, C, D consistently show only major peaks at 83ºC.

The Ct values obtained from quantitative expression of *PGSC0003DMG400003985* gene in samples at all stages of 6 cultivars varied from 17.65 to 27.29 (appendix D.1.1).

4.3.1.2 Expression of *PGSC0003DMG400023270* gene, putatively encoding elongation factor 1-α

The standard curve indicates that Ct values of duplicates of each concentration of cDNA input, ranging from 0.03ng to 300ng, were within the range of the standard curve (figure 16). The analysis indicates the correlation coefficient (R^2) of 0.997, the lope of -3,358 in the range of successful reactions (between -3.58 and -3.10) and the amplification efficiency of 98.5% in the range of successful reactions (between 90% and 110%).

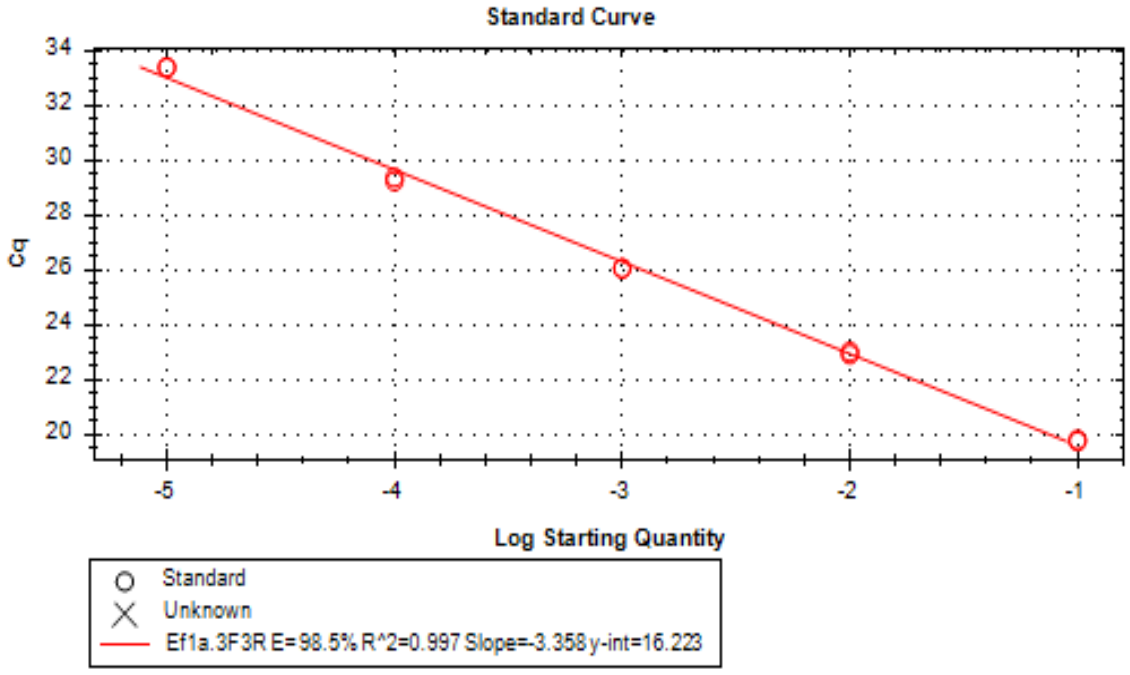


Figure 16: Regression line for quantity of primer amplification efficiencies and melting curve analysis from quantitative expression of *PGSC0003DMG400023270* gene, putatively encoding elongation factor 1-α, in stage-1 infected sample of cultivar Beate with template cDNA input in the range of 0.03ng to 300ng. The figure indicates: Correlation coefficient (R^2) = 0.997. Slope = -3,358. Efficiency E = 98,5%.

The melt curve analysis of quantitative expression of the gene in samples of four stages in tuber development consistently show only 1 peak at 81.5°C (figure 17A, B, C, and D).

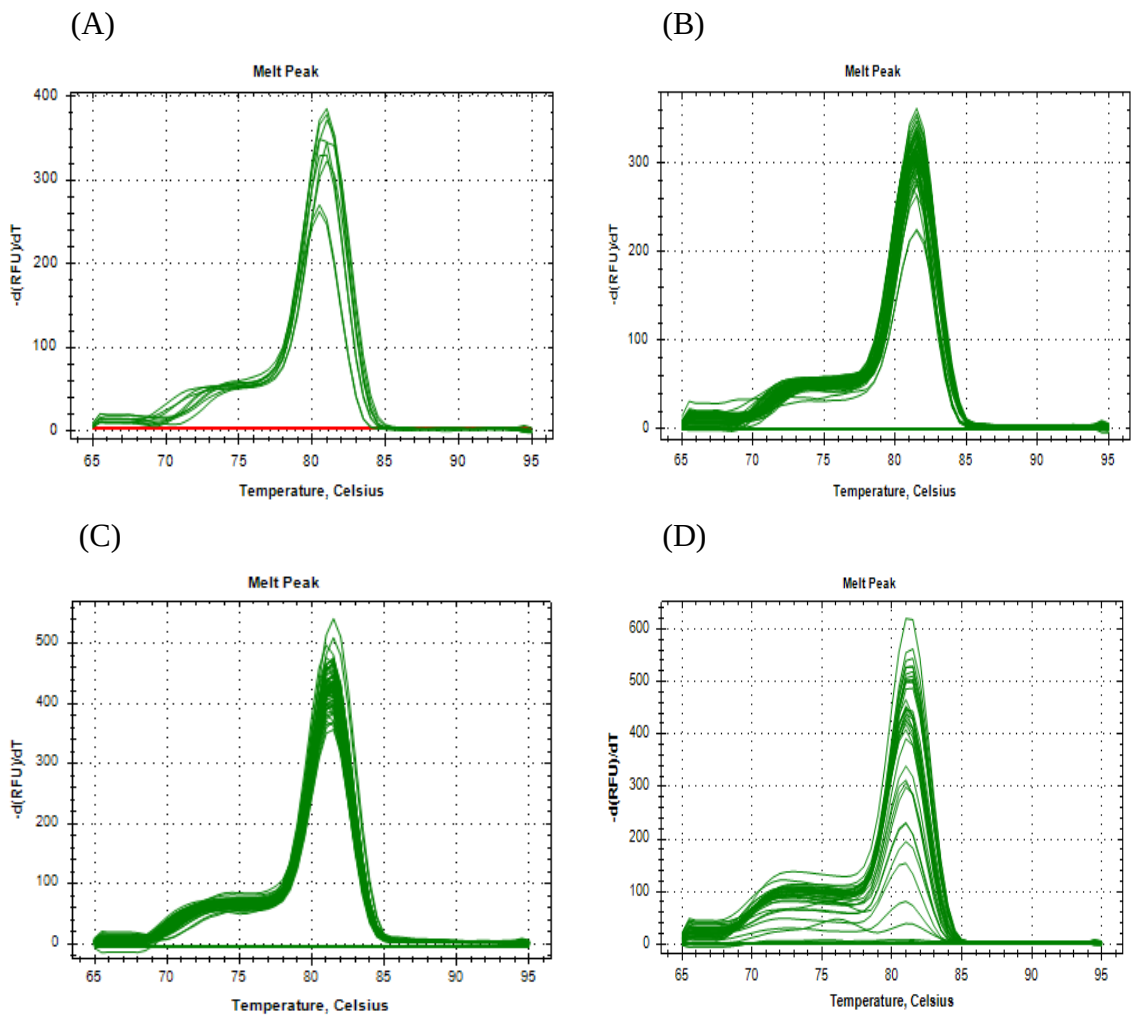


Figure 17: The dissociation (melting) curve analyses from quantitative expression of *PGSC0003DMG400023270* gene, putatively encoding elongation factor 1-α, were conducted to identify amplicons produced during PCR. (A) Stage-1 infected sample of cultivar Beate with template cDNA input in the range of 0.03ng to 300ng. (B) Stage-1 infected and non-infected samples of 6 cultivars with template cDNA input of 300ng. (C) Stage-2 infected and non-infected samples of 6 cultivars with template cDNA input of 300ng. (D) Stage-3 and stage-4 infected samples of 3 cultivars with template cDNA input of 300ng. Figures A, B, C, D consistently show only major peaks at 81.5^{o}C.

The Ct values obtained from quantitative expression of *PGSC0003DMG400023270* gene in samples at all stages of 6 cultivars varied from 16.6 to 28.66 (appendix D1.2).

4.3.1.3 Expression of *PGSC0003DMG400044276* gene, putatively encoding 18SrRNA

The standard curve indicates that Ct values of duplicates of each concentration of cDNA input, ranging from 0.03ng to 300ng, were within the range of the standard curve (figure 18). The analysis indicates the correlation coefficient (R^2) of 0.999, the lope of -3,413 in the range of successful reactions (between -3.58 and -3.10) and the amplification efficiency of 96.3% in the range of successful reactions (between 90% and 110%).

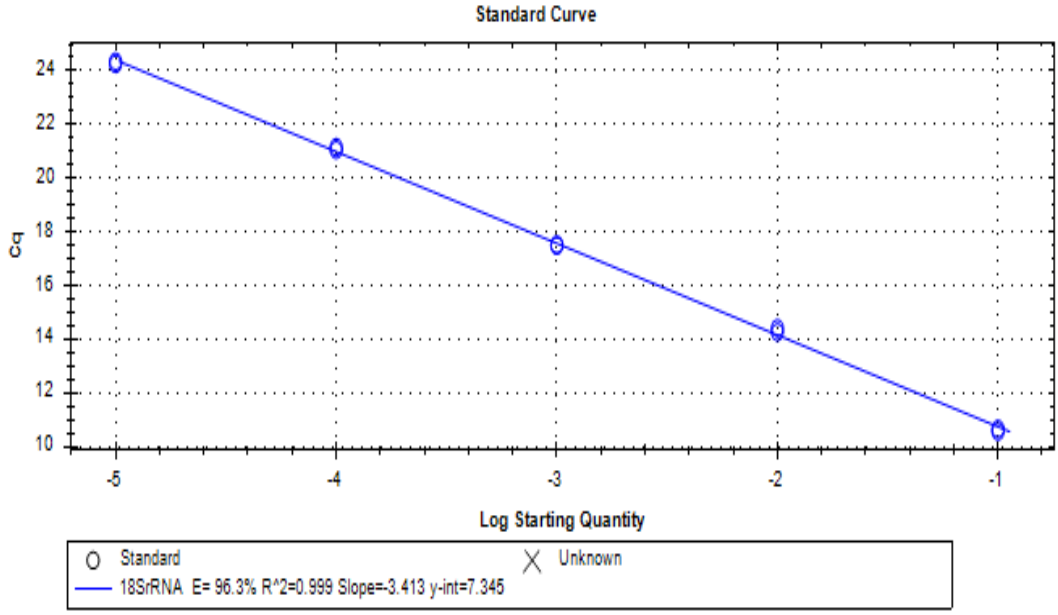


Figure 18: Regression line for quantity of primer amplification efficiencies and melting curve analysis from quantitative expression *PGSC0003DMG400044276* gene, putatively encoding 18S rRNA in stage-1 sample of cultivar Beate with template cDNA input in the range of 0.03ng to 300ng. The figure indicates: Correlation coefficient (R^2) = 0.999. Slope = -3,413. Efficiency E = 96.3%.

The melt curve analysis of quantitative expression of the gene in samples four stages in tuber development show the major peak at 88^{o}C (figure 19A, B, C, and D). Although the melt curves of template cDNA input in the range of 0.03ng to 300ng shows only major peak at 88^{o}C (figure 19A), melt curves of stage-1 infected and non-infected samples of 6 cultivars with template cDNA input of 300ng shows major peak at 88^{o}C and another peak at 80^{o}C (figure 19B) and similar with stage-3 and stage-4 samples (19D). Whereas, melt curves of

stage-2 infected and non-infected samples of 6 cultivars with template cDNA input of 300ng shows major peak at 88°C and other peaks in the range of 80°C to 83 °C (figure 19C).

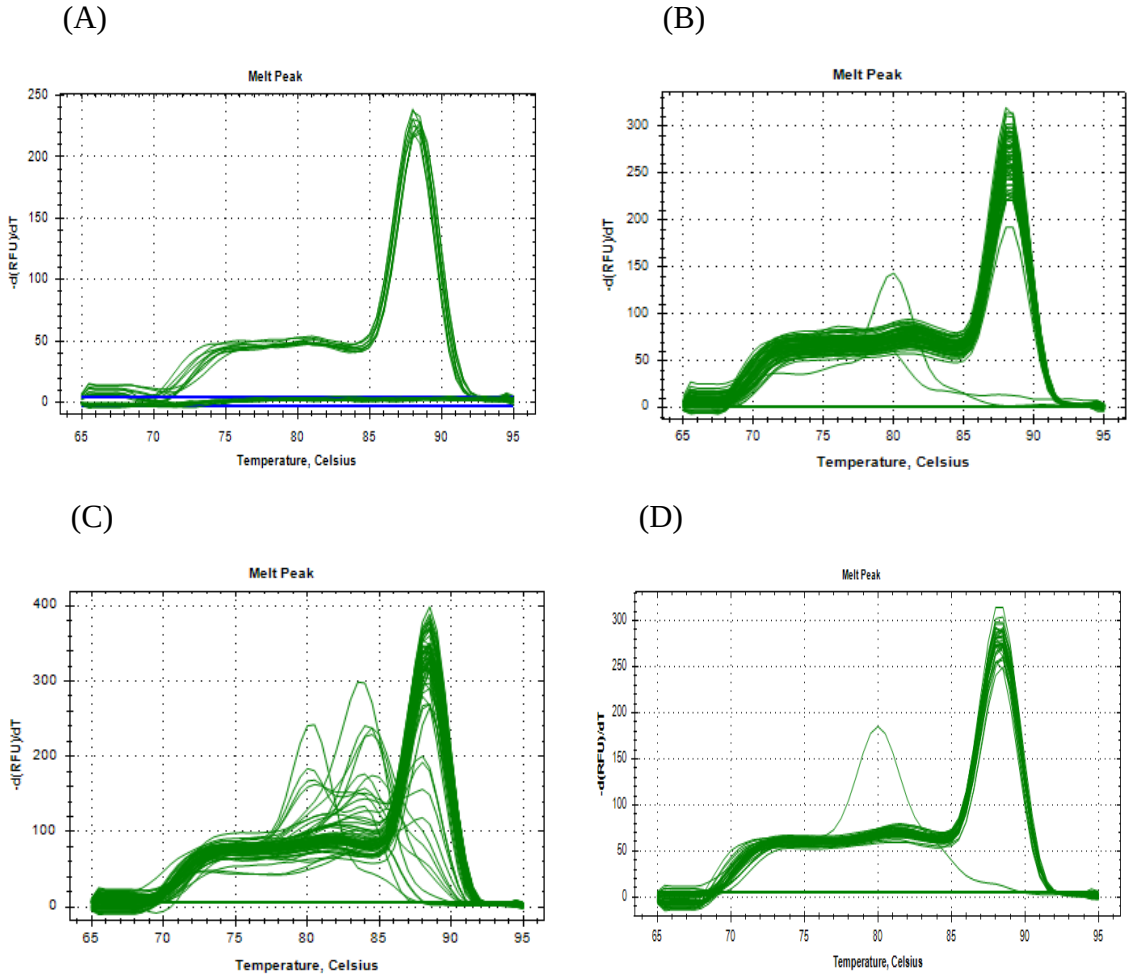


Figure 19: The dissociation (melting) curve analyses from quantitative expression of *PGSC0003DMG400044276* gene, putatively encoding 18SrRNA, were conducted to identify amplicons produced during PCR. (A) Stage-1 infected sample of cultivar Beate with template cDNA input in the range of 0.03ng to 300ng, shows only major peak at 88°C. (B) Stage-1 infected and non-infected samples of 6 cultivars with template cDNA input of 300ng shows major peak at 88°C and another peak at 80°C. (C) Stage-2 infected and non-infected samples of 6 cultivars with template cDNA input of 300ng. Figure C shows major peak at 88°C, multiple peaks in the range of 80°C to 83 °C. (D) Stage-3 and stage-4 infected samples of 3 cultivars with template cDNA input of 300ng. Figure D shows major peak at 88°C and another peak at 80°C.

The Ct values obtained from quantitative expression of *PGSC0003DMG400044276* gene in samples at all stages of 6 cultivars varied from 10.82 to 37.35 (appendix D.1.3).

4.3.2 Expression of genes uniquely expressed in the resistant cultivar Beate

To investigate the correlation between the expression of the candidate genes and the infection caused by *S. turgidiscabies*, expression pattern of each investigated gene using real-time PCR was compared with its expression pattern using RNA-sequencing in cultivar Beate and cultivar Saturna when they were infected with *S. turgidiscabies* isolates.

To investigate the correlation between the expression of the candidate genes and the resistance to common scab disease, expression patterns of each investigated gene by real-time PCR were compared within 6 cultivars. The results from infection experiment on potato plants indicated that cultivar Beate and cultivar Tivoli are resistant to *S. turgidiscabies* (as discussed in section 5.2). Therefore, expression patterns of cultivar Beate and cultivar Tivoli by real-time PCR were compared. On the other hand, cultivar DSxAS, cultivar Gullauge, cultivar Pimpernel, and cultivar Saturna were indicated to be susceptible to *S. turgidiscabies* by the infection experiment on potato plants, thus, their expression patterns were also compared.

Abbreviations of samples presented in all gene expression results are explained as below:

Be: Beate non-infected samples	BeSt: Beate *S. turgidiscabies*-infected samples
Ds: DSxAS non-infected samples	DsSt: DSxAs *S. turgidiscabies*-infected samples
Gu: Gullauge non-infected samples	GuSt:Gullauge *S. turgidiscabies*-infected samples
Pi: Pimpernel non-infected samples	PiSt:Pimpernel *S. turgidiscabies*-infected samples
Sa: Saturna non-infected samples	SaSt: Saturna *S. turgidiscabies*-infected samples
Ti: Tivoli non-infected samples	TiSt: Tivoli *S. turgidiscabies*-infected samples

4.3.2.1 Expression of gene *PGSC0003DMG400040744*, putatively coding for late blight resistance protein

Expression patterns of this gene in RNA-sequencing and real-time PCR methods were inconsistent. RNA-sequencing data described unique expression of this gene in cultivar Beate, particularly high expression was shown in stage-1 infected samples (figure 20A). Inconsistently, real-time PCR data indicated that this gene up-regulated in infected samples of both cultivar Beate, and cultivar Saturna, and down-regulated in non-infected samples (figure 20B).

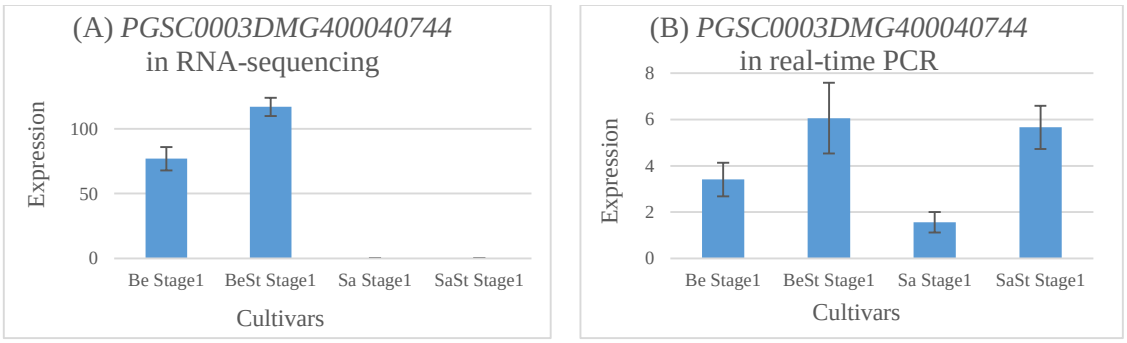


Figure 20: The comparison of expression of *PGSC0003DMG400040744* gene, putatively coding for late blight resistant protein, at stage-1 non-infected and infected samples of cultivar Beate and cultivar Saturna (A) using RNA-sequencing (RNA-sequencing data were supplied by NIBIO) and (B) using real-time PCR. RNA samples used in these methods were obtained from two independent experiments infected with the same *S. turgidiscabies* isolates.

Compared expression of this gene in 6 cultivars, real-time PCR data generally performed up-regulated expression patterns in cultivar Beate, cultivar DSxAs and cultivar Saturna. Reversely, it was down-regulated in cultivar Gullauge, cultivar Pimpernel and cultivar Tivoli (figure 21).

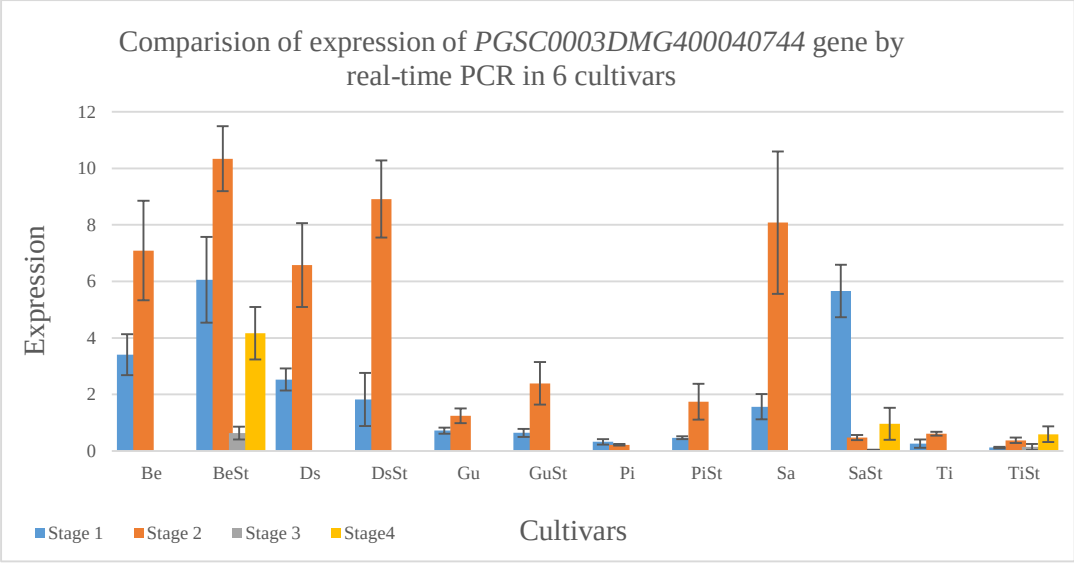


Figure 21: Comparison of expression of *PGSC0003DMG400040744* gene at stage-1, stage-2, stage-3 and stage-4 samples of 6 cultivars by real-time PCR. Data presented in each bar are mean expression value of 3 cDNA samples collected from 3 different potato plants of the same cultivar (presented in appendix B). (The Ct values of gene expression presented in appendix D.2.1).

No similarity in expression patterns of two resistant cultivars, cultivar Beate and cultivar Tivoli, was shown. Differences in expression patterns of 4 susceptible cultivars were shown. In which, the gene was up-regulated in cultivar DSxAs and cultivar Saturna, while it was down-regulated in cultivar Gullauge and cultivar Pimpernel.

4.3.2.2 Expression of gene *PGSC0003DMG400017087*, putatively encoding late blight resistance protein homologue r1a-10

Expression patterns of this gene in RNA-sequencing and real-time PCR methods were consistent. RNA-sequencing data described unique expression of this gene in cultivar Beate, particularly up-regulated expression was shown in stage-1 infected samples (figure 22A). Consistently, real-time PCR data indicated that this gene was up-regulated in infected samples of cultivar Beate, around 2 fold up-regulated; but only around 0.5 fold expression at stage-1 non-infected samples in cultivar Beate. Whereas, very low expression in infected samples and non-infected samples of cultivar Saturna (figure 22B).

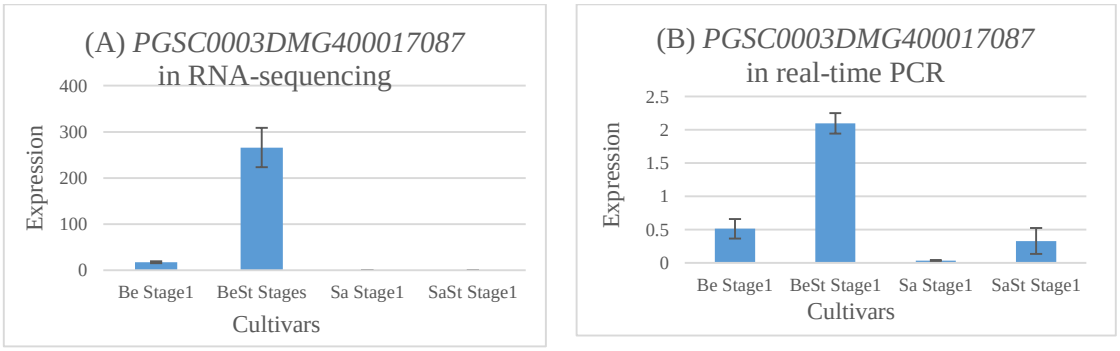


Figure 22: comparison of expression of *PGSC0003DMG400017087*, putatively encoding late blight resistance protein homologue r1a-10, at stage-1 non-infected and infected samples of cultivar Beate and cultivar Saturna (A) using RNA-sequencing (RNA-sequencing data were supplied by NIBIO) and (B) using real-time PCR. RNA samples used in these methods were obtained from two independent experiments infected with the same *S. turgidiscabies* isolates.

Compared expression of this gene in 6 cultivars, real-time PCR data performed up-regulated expression patterns in cultivar DSxAs, up to ten fold and cultivar Beate, up to around 5 fold. Reversely, it was down-regulated in cultivar Gullauge, cultivar Pimpernel, cultivar Saturna and cultivar Tivoli (figure 23). No similarity in expression patterns of two resistant cultivars, cultivar Beate and cultivar Tivoli, was shown. Differences in expression patterns of 4

susceptible cultivars were shown. In which, the gene was up-regulated in cultivar DSxAs, while it was down-regulated in cultivar Gullauge, cultivar Pimpernel and cultivar Saturna.

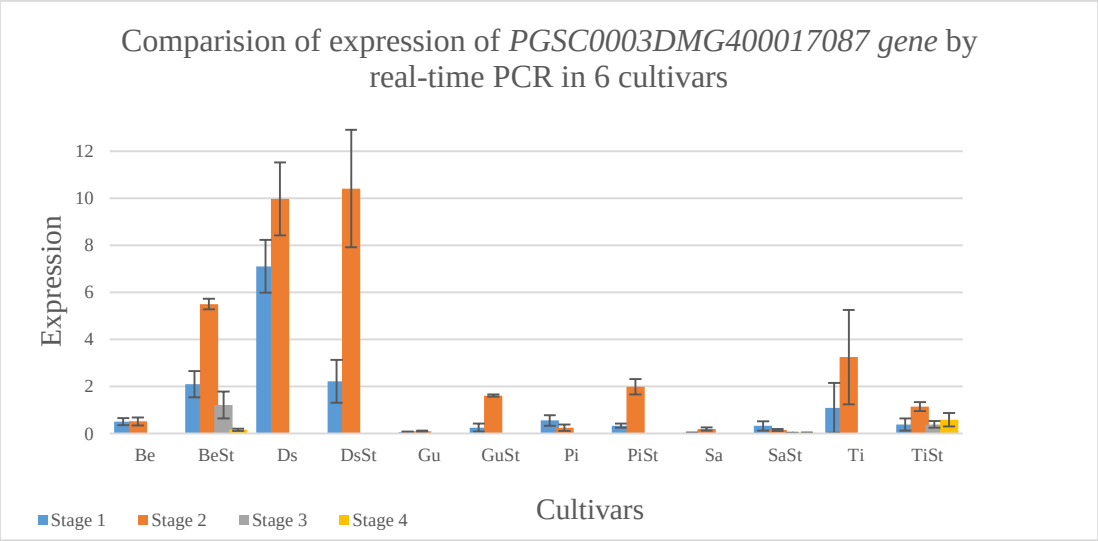


Figure 23: Comparison of expression of *PGSC0003DMG400017087*, putatively encoding late blight resistance protein homologue r1a-10 at stage-1, stage-2, stage-3 and stage-4 samples of 6 cultivars by real-time PCR. Data presented in each bar are mean expression value of 3 cDNA samples collected from 3 different potato plants of the same cultivar (presented in appendix B). (The Ct values of gene expression presented in appendix D.2.2).

4.3.2.3 Expression of gene *PGSC0003DMG400007385*, putatively encoding disease resistant protein At4g27190

Expression patterns of this gene in RNA-sequencing and real-time PCR methods were consistent. RNA-sequencing data described unique expression of this gene in cultivar Beate, up-regulated expression were shown in stage-1 infected and non-infected samples (figure 24A). Consistently, real-time PCR data indicated that this gene expressed in infected samples of cultivar Beate, around 0.7 fold; and only around 0.4 fold expression at stage-1 non-infected samples in cultivar Beate. Whereas, very low expression in infected samples and non-infected samples of cultivar Saturna (figure 24B).

Compared expression of this gene in 6 cultivars, real-time PCR data performed up-regulated expression patterns in stage-2 non-infected samples of cultivar Beate and cultivar DSxAs, particularly it was also up-regulated in stage-4 infected samples of cultivar DSxAs. Reversely, it was down-regulated in cultivar Gullauge, cultivar Pimpernel, and cultivar Saturna and very low regulated in cultivar Tivoli (figure 25). No similarity in expression

patterns of two resistant cultivars, cultivar Beate and cultivar Tivoli, was shown. Differences in expression patterns of 4 susceptible cultivars were described. In which, the gene was up-regulated in cultivar DSxAs, while it was down-regulated in cultivar Gullauge, cultivar Pimpernel and cultivar Saturna.

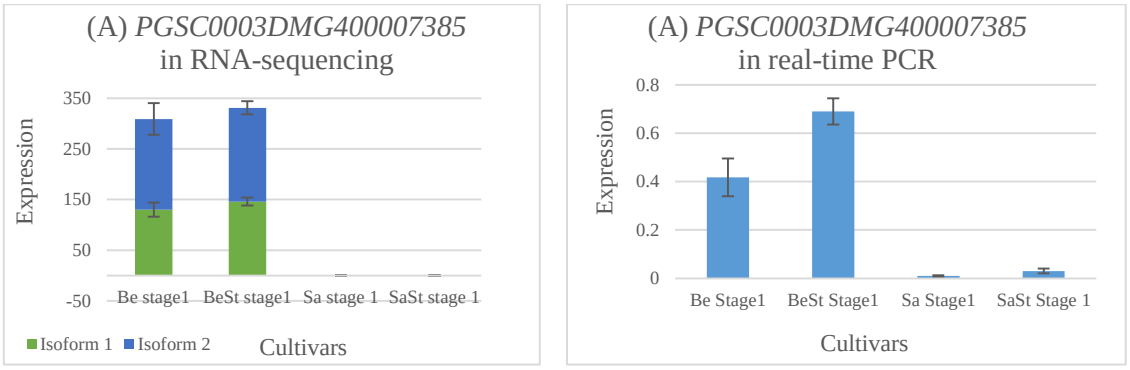


Figure 24: comparison of expression of *PGSC0003DMG400007385*, putatively encoding disease resistant protein At4g27190, at stage-1 non-infected and infected samples of cultivar Beate and cultivar Saturna (A) using RNA-sequencing (RNA-sequencing data were supplied by NIBIO) and (B) using real-time PCR. RNA samples used in these methods were obtained from two independent experiments infected with the same *S. turgidiscabies* isolates.

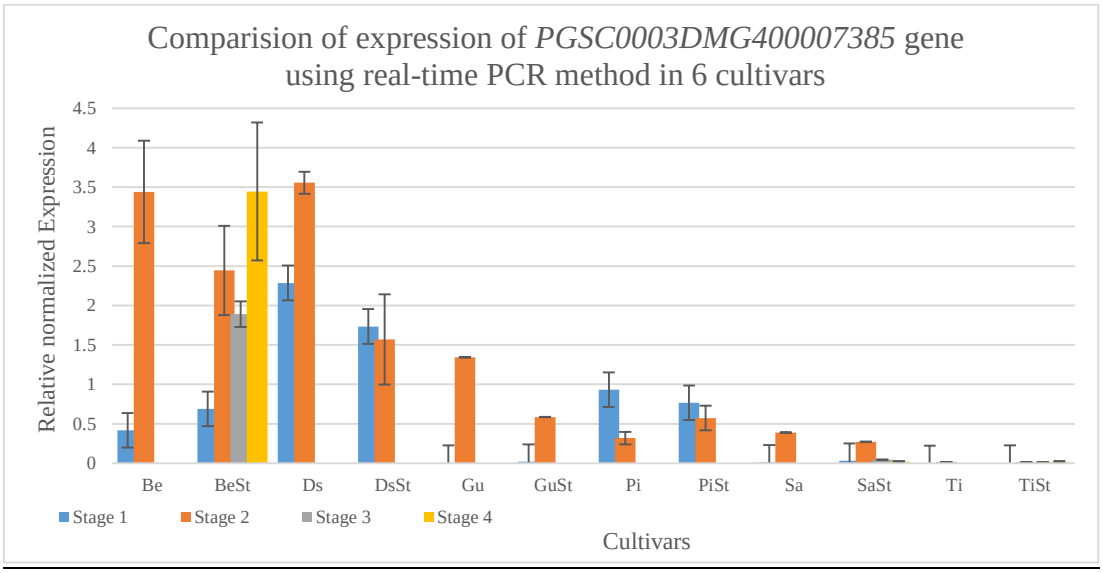


Figure 25: Comparison of expression of *PGSC0003DMG400007385*, putatively encoding disease resistant protein At4g27190 in stage-1, stage-2, stage-3 and stage-4 samples of 6 cultivars using real-time PCR method. Data presented in each bar are mean expression value of 3 cDNA samples collected from 3 different potato plants of the same cultivar (presented in appendix B). (The Ct values of gene expression presented in appendix D.2.3).

4.3.2.4 Expression of gen e *PGSC0003DMG400010612*, putatively coding for disease resistant protein rpp13

Expression patterns of this gene in RNA-sequencing and real-time PCR methods were consistent. RNA-sequencing data described unique expression of this gene in cultivar Beate, up-regulated expression were shown in stage-1 infected samples (figure 26A). Consistently, real-time PCR data shown higher expression in infected samples of cultivar Beate, nearly 4 fold; and lower expression in stage-1 non-infected samples of cultivar Beate, nearly 3 fold. Whereas, under 1 fold expression in infected samples and non-infected samples of cultivar Saturna were shown (figure 26B).

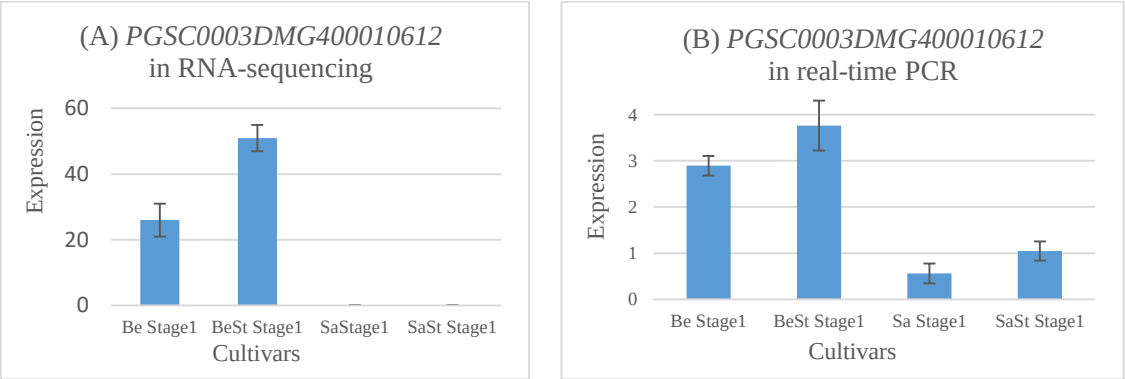


Figure 26: comparison of expression of *PGSC0003DMG400010612*, putatively coding for disease resistant protein rpp13, at stage-1 non-infected and infected samples of cultivar Beate and cultivar Saturna (A) using RNA-sequencing (RNA-sequencing data were supplied by NIBIO) and (B) using real-time PCR. RNA samples used in these methods were obtained from two independent experiments infected with the same *S. turgidiscabies* isolates.

Compared expression of this gene in 6 cultivars, real-time PCR data performed tenfold up-regulated expression in stage-2 infected samples of cultivar Beate and above fourfold expression in stage-2 infected samples of this cultivar. Reversely, similar expression patterns were described in susceptible cultivars. Low expression were seen in cultivar DSxAs, cultivar Gullauge and cultivar Saturna; and very low expression in cultivar Pimpernel and cultivar Tivoli (figure 27). No similarity in expression patterns of two resistant cultivars, cultivar Beate and cultivar Tivoli, was shown.

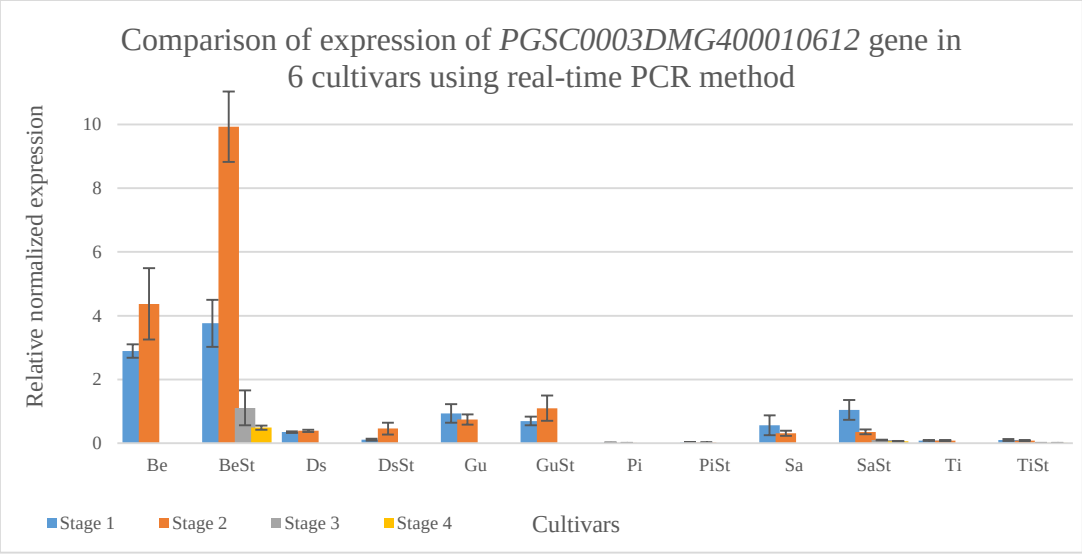


Figure 27: Comparison of expression of *PGSC0003DMG400010612*, putatively coding for disease resistant protein rpp13 in stage-1, stage-2, stage-3 and stage-4 samples of 6 cultivars using real-time PCR method. Data presented in each bar are mean expression value of 3 cDNA samples collected from 3 different potato plants of the same cultivar (presented in appendix B). (The Ct values of gene expression presented in appendix D.2.4).

4.3.2.5 Expression of gene *PGSC0003DMG400013405*, putatively encoding *myb*-related protein myb4

Expression patterns of this gene in RNA-sequencing and real-time PCR methods were inconsistent. RNA-sequencing data described unique expression of this gene in cultivar Beate, particularly high expression was shown in stage-1 infected samples (figure 28A). Inconsistently, real-time PCR data shown higher expression in infected samples of cultivar Saturna, and low expression in non-infected samples of cultivar Saturna as well as non-infected and infected samples of Beate (figure 28B).

Compared expression of this gene in 6 cultivars, real-time PCR data performed highest expression in stage-1 infected samples of cultivar Saturna, above fivefold, even higher than that in cultivar Beate and cultivar Tivoli. High expression were seen in stage-2 non-infected samples of cultivar Saturna and cultivar Tivoli. Reversely, low expression patterns were described in susceptible cultivars Gullauge and cultivar Pimpernel (figure 29). No similarity in expression patterns of two resistant cultivars, cultivar Beate and cultivar Tivoli, was shown.

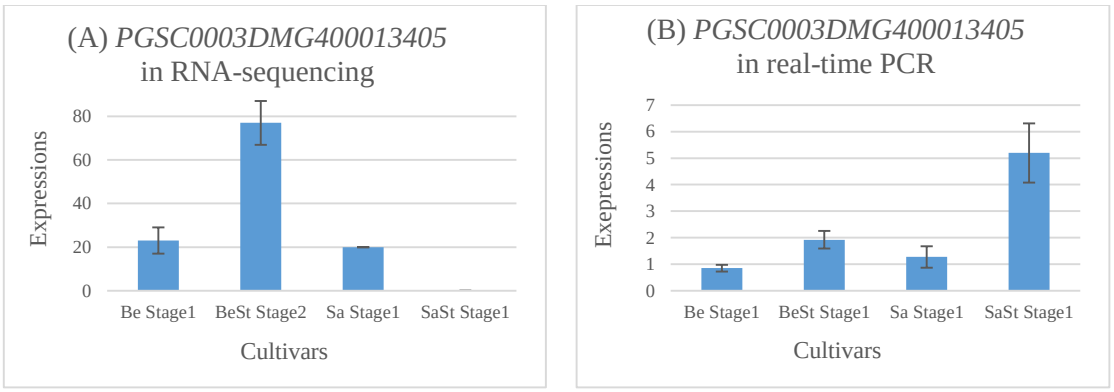


Figure 28: comparison of expression of *PGSC0003DMG400013405*, putatively encoding myb-related protein myb4, at stage-1 non-infected and infected samples of cultivar Beate and cultivar Saturna (A) using RNA-sequencing (RNA-sequencing data were supplied by NIBIO) and (B) using real-time PCR. RNA samples used in these methods were obtained from two independent experiments infected with the same *S. turgidiscabies* isolates.

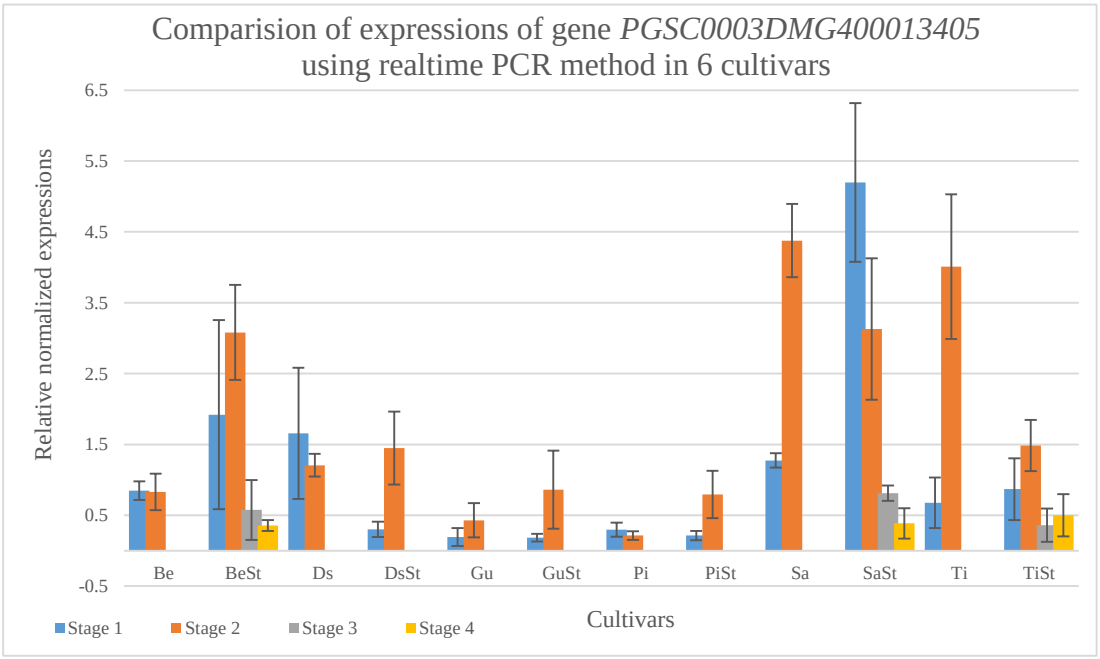


Figure 29: Comparison of expression of *PGSC0003DMG400013405*, putatively encoding myb-related protein myb4 in stage-1, stage-2, stage-3 and stage-4 samples of 6 cultivars using real-time PCR method. Data presented in each bar are mean expression value of 3 cDNA samples collected from 3 different potato plants of the same cultivar (presented in appendix B). (The Ct values of gene expression presented in appendix D.2.5).

4.3.2.6 Expression of gene *PGSC0003DMG400000655*, putatively coding serine threonine-protein phosphatase-7

RNA-sequencing data performed expression of 4 different alternative spliced transcripts of this gene, which uniquely expressed in cultivar Beate with higher expression in infected samples (figure 30A). Real-time PCR data also performed the unique expression pattern of this gene in cultivar Beate. However, the higher expression was observed in non-infected samples, around 1.2 relative normalized folds. Whereas, it was only around 0.6 relative normalized fold in infected samples (figure 30B).

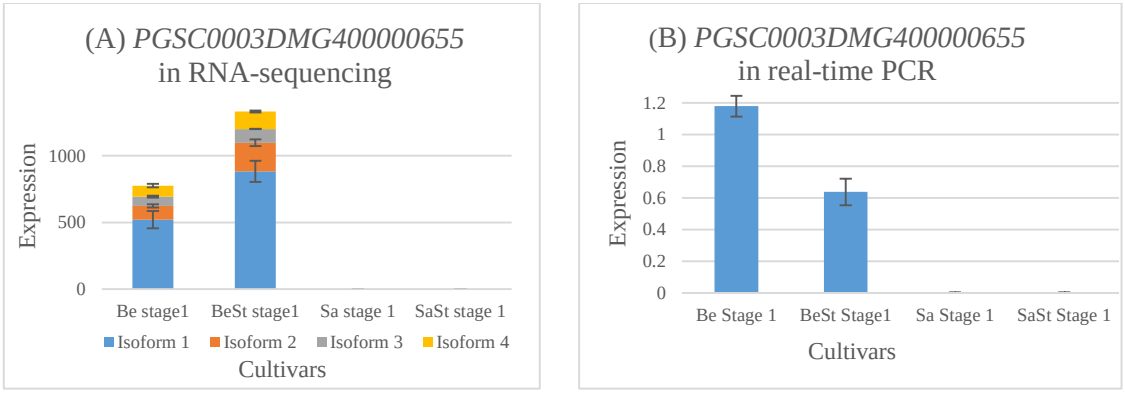


Figure 30: comparison of expression of gene *PGSC0003DMG400000655*, putatively coding serine threonine-protein phosphatase-7, at stage-1 non-infected and infected samples of cultivar Beate and cultivar Saturna (A) using RNA-sequencing (RNA-sequencing data were supplied by NIBIO) and (B) using real-time PCR. RNA samples used in these methods were obtained from two independent experiments infected with the same *S. turgidiscabies* isolates.

Compared expression of this gene in 6 cultivars, real-time PCP data indicated that the gene expressed in all cultivars. In cultivar Beate, the gene performed higher expression pattern in non-infected tage-1 samples than that in infected stage-1 samples. The gene performed highest expression pattern in stage-4 infected samples of Beate, up to 5 folds. No similarity in expression patterns of two resistant cultivars, cultivar Beate and cultivar Tivoli, was shown (figure 31).

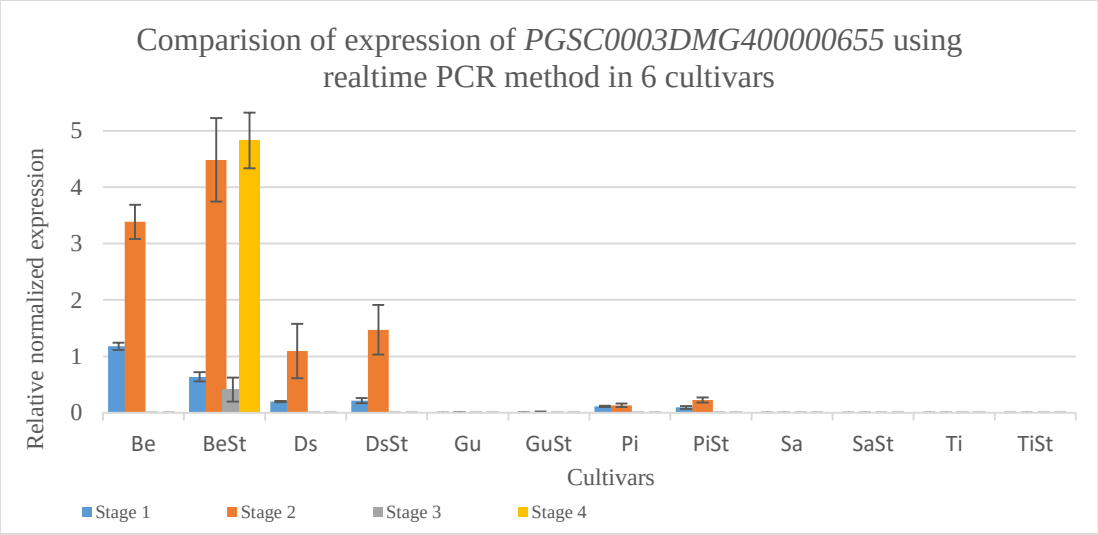


Figure 31: Comparison of expression of gene *PGSC0003DMG400000655*, putatively coding serine threonine-protein phosphatase-7 in stage-1, stage-2, stage-3 and stage-4 samples of 6 cultivars using real-time PCR method. Data presented in each bar are mean expression value of 3 cDNA samples collected from 3 different potato plants of the same cultivar (presented in appendix B). (The Ct values of gene expression presented in appendix D.2.6).

4.3.2.7 Expression of gene *PGSC0003DMG400015425*, putatively encoding for sre1a protein

RNA sequencing data performed 6 different alternative spliced transcripts of this gene, in which, isoform 3 uniquely expressed in cultivar Beate. Accumulated expression of 6 isoforms in cultivar Beate and 5 isoforms in cultivar Saturna described the highest expression in stage-1 infected samples of cultivar Saturna. High expression also performed in stage-1 infected samples of cultivar Beate (figure 32A). However, real-time PCR data performed higher expression in infected as well as non-infected samples of cultivar Beate; and lower expression in non-infected and infected samples of cultivar Saturna (figure 32B).

Compared expression of this gene in 6 cultivars, real-time PCR data shown almost even expression, ranging from 1 to 2 fold, in stage-1 samples of all cultivars. In cultivar Beate, cultivar DSxAs, cultivar Gullauge and cultivar Tivoli, the expression in non-infected samples were higher than those in infected samples. In cultivar Beate, the gene expressed up to around fivefold in stage-2 non-infected samples and tenfold in infected samples. In Tivoli, also expressed at 4 fold level in stage-2 non-infected samples and around 1 fold in stage-2

infected samples (figure 33). In general, no similarity in expression patterns of two resistant cultivars, cultivar Beate and cultivar Tivoli, was shown.

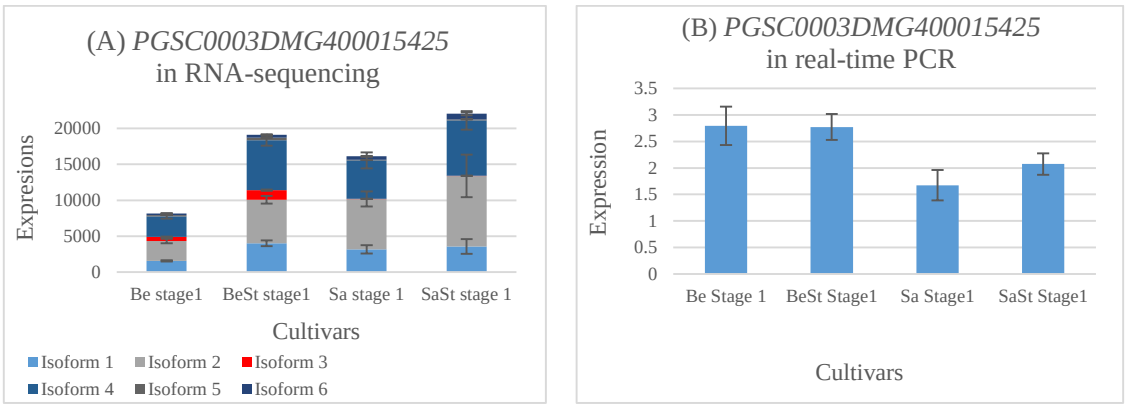


Figure 32: comparison of expression of gene *PGSC0003DMG400015425*, putatively encoding sre1a protein, at stage-1 non-infected and infected samples of cultivar Beate and cultivar Saturna (A) using RNA-sequencing (RNA-sequencing data were supplied by NIBIO) and (B) using real-time PCR. RNA samples used in these methods were obtained from two independent experiments infected with the same *S. turgidiscabies* isolates.

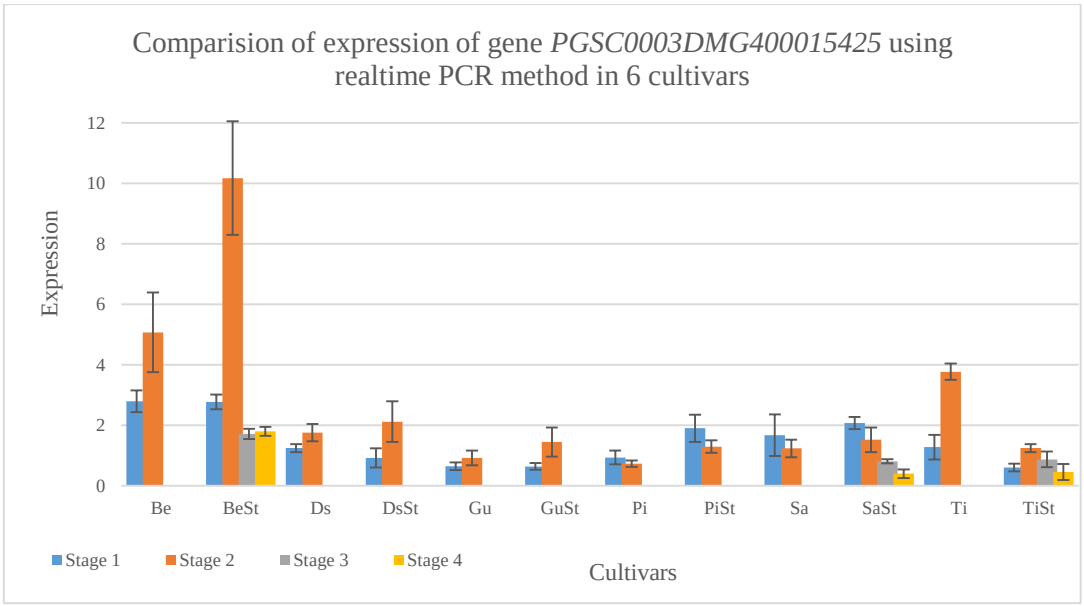


Figure 33: Comparison of expression of gene *PGSC0003DMG400015425*, putatively encoding sre1a protein, in stage-1, stage-2, stage-3 and stage-4 samples of 6 cultivars using real-time PCR method. Data presented in each bar are mean expression value of 3 cDNA samples collected from 3 different potato plants of the same cultivar (presented in appendix B). (The Ct values of gene expression presented in appendix D.2.7).

4.3.3 Expression of putative candidate thaxtomin A-associated genes

4.3.3.1 Expression of gene *PGSC0003DMG400014859*, putatively encoding trx1-like protein

Expression patterns of this gene in RNA-sequencing and real-time PCR methods were almost consistent. RNA-sequencing data described expression of this gene in both cultivar Beate and cultivar Saturna. The higher expression pattern was shown in cultivar Beate (figure 34A). Real-time PCR data indicated that this gene evenly expressed in both non-infected and infected samples of both cultivar Beate and cultivar Saturna (figure 34B).

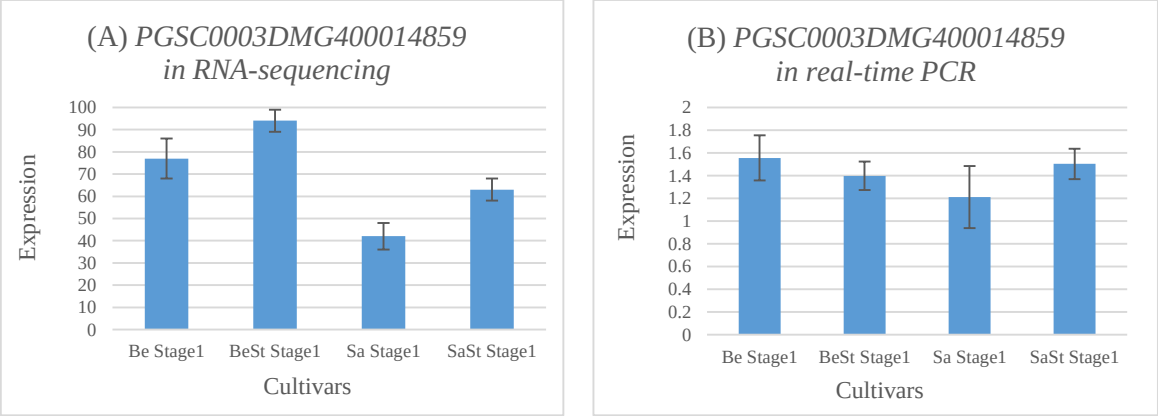


Figure 34: comparison of expression of gene *PGSC0003DMG400014859*, putatively encoding trx1-like protein, at stage-1 non-infected and infected samples of cultivar Beate and cultivar Saturna (A) using RNA-sequencing (RNA-sequencing data were supplied by NIBIO) and (B) using real-time PCR. RNA samples used in these methods were obtained from two independent experiments infected with the same *S. turgidiscabies* isolates.

Compared expression of this gene in 6 cultivars, Real-time PCR data generally performed similar expression in stage-1 samples of all cultivars, regardless of non-infected and infected samples. Higher expression was observed in stage-2 samples of all cultivars, regardless of resistance and susceptibility to common scab (figure 35).

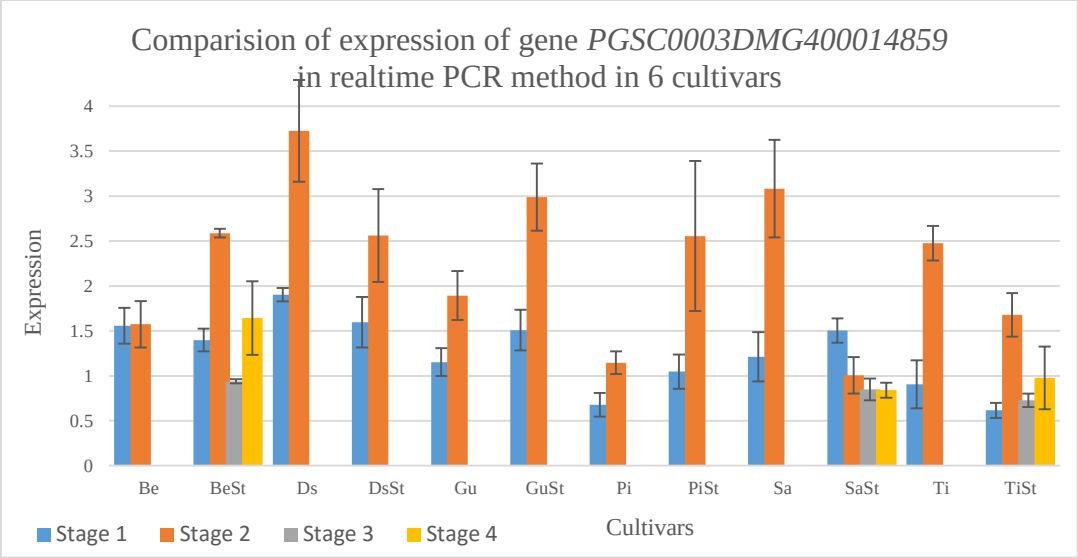


Figure 35*:* Comparison of expression of *PGSC0003DMG400014859,* putatively encoding trx1-like protein, in stage-1, stage-2, stage-3 and stage-4 samples of 6 cultivars using real-time PCR method. Data presented in each bar are mean expression value of 3 cDNA samples collected from 3 different potato plants of the same cultivar (presented in appendix B). (The Ct values of gene expression presented in appendix D.3.1).

4.3.3.2 Expression of gene *PGSC0003DMG400025578,* putatively encoding trx1-like protein

Overall, the expression of this gene in both methods are consistent. RNA sequencing data indicated 2 alternative spliced transcripts of this gene, in which isoform 1 uniquely expressed in cultivar Beate, whereas, isoform 2 expressed in both cultivar Beate and cultivar Saturna. Ratio of isoform 1: isoform 2 expression in cultivar Beate was 7:10. Overall, accumulated expression levels of these 2 isoforms in cultivar Beate were similar to the expression of isoform 2 in cultivar Saturna (figure 36A). Real-time PCR data performed an insignificant difference in expression patterns of these two cultivars (figure 36B).

Compared expression of this gene in 6 cultivars, real-time PCR data generally performed a higher expression pattern in stage-1 samples of cultivar Beate and cultivar Saturna. Likewise, the gene performed higher expression in stage 2 samples of cultivar Tivoli. No significant differences in expression of non-infected and infected samples were shown as well as no differences in expression of resistant cultivars and susceptible cultivars were described (figure 37).

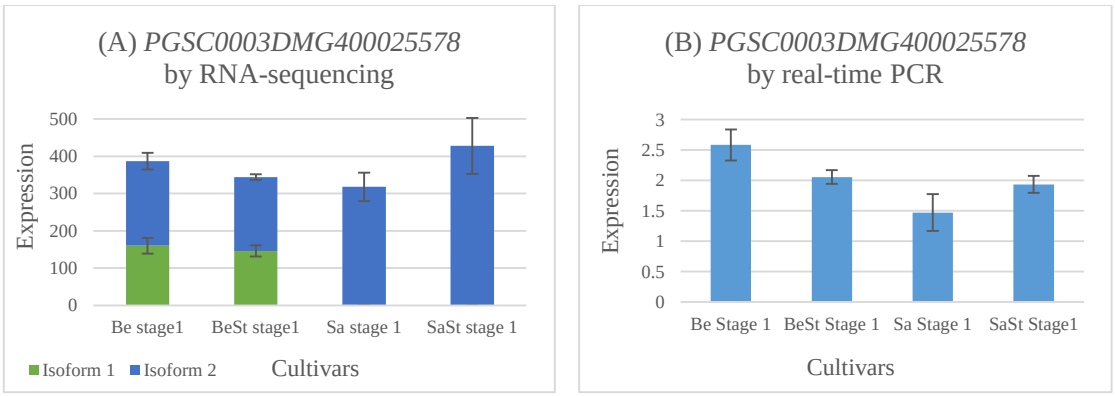


Figure 36: comparisons of expression of *PGSC0003DMG400025578* putatively encoding trx1-like protein, at stage-1 non-infected and infected samples of cultivar Beate and cultivar Saturna (A) using RNA-sequencing (RNA-sequencing data were supplied by NIBIO) and (B) using real-time PCR. RNA samples used in these methods were obtained from two independent experiments infected with the same *S. turgidiscabies* isolates.

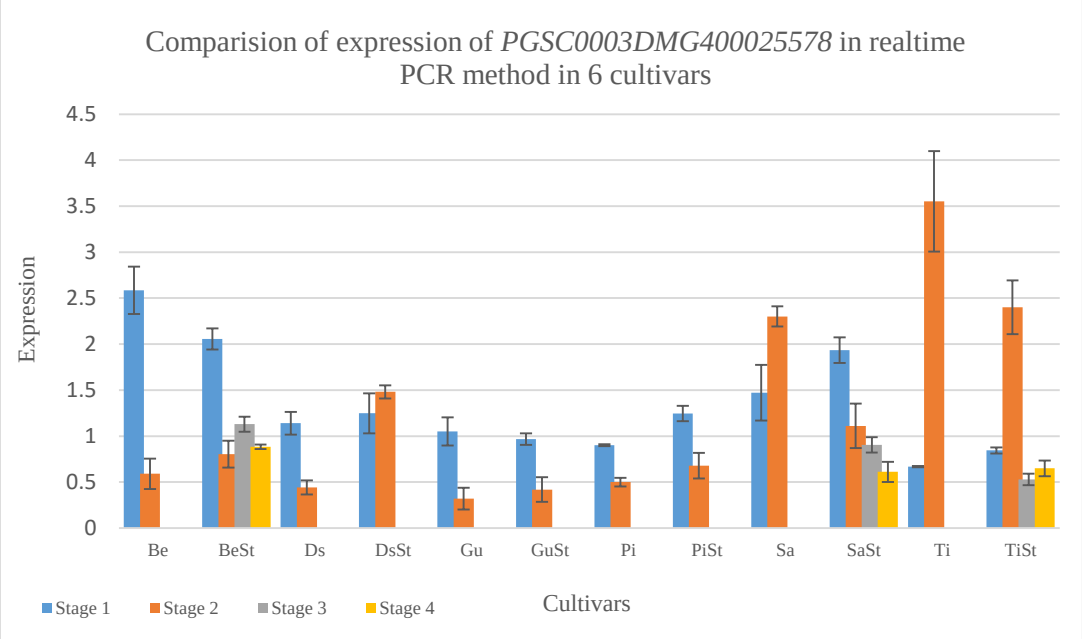


Figure 37: Comparison of expression of *PGSC0003DMG400025578*, putatively encoding trx1-like protein, in stage-1, stage-2, stage-3 and stage-4 samples of 6 cultivars using real-time PCR method. Data presented in each bar are mean expression value of 3 cDNA samples collected from 3 different potato plants of the same cultivar (presented in appendix B). (The Ct values of gene expression presented in appendix D.3.2).

4.3.3.3 Expression of gene *PGSC0003DMG402017989*, putatively encoding cyclic nucleotide-gated ion channel 1-like protein

Overall, this gene performed consistent expression patterns in both methods. RNA-sequencing data indicated 7 alternative spliced transcripts of the gene. The accumulated expression pattern of this gene described the up-regulations in infected samples of both cultivars and higher in cultivar Saturna (figure 38A). Realtime-PCR also described the up-regulations of this gene in infected samples of both cultivars, at the same level in both cultivars at around 1.7 normalized fold; and down-regulations in non-infected samples at the same level in both cultivars at around 0.5 normalized fold (figure 38B).

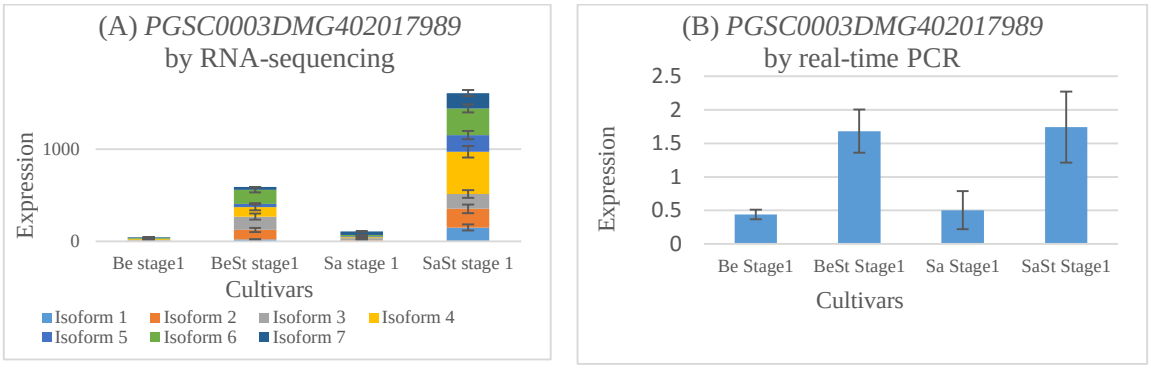


Figure 38: comparisons of expression of *PGSC0003DMG402017989* putatively encoding cyclic nucleotide-gated ion channel 1-like protein, at stage-1 non-infected and infected samples of cultivar Beate and cultivar Saturna (A) using RNA-sequencing (RNA-sequencing data were supplied by NIBIO) and (B) using real-time PCR. RNA samples used in these methods were obtained from two independent experiments infected with the same *S. turgidiscabies* isolates.

Compared expression of this gene in 6 cultivars, real-time PCR data generally described higher expression in stage-1 infected samples than those in non-infected samples of all cultivars. Overall, the gene was higher expressed in stage-2 samples of cultivar Beate, cultivar DSxAs and cultivar Pimpernel. No similarity in expression of resistant cultivars and susceptible cultivars were described (figure 39).

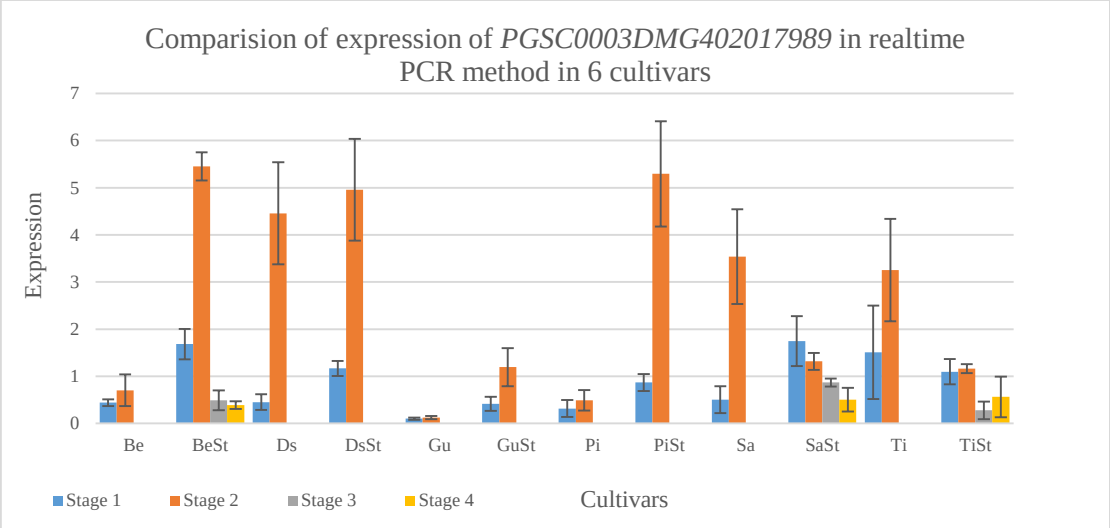


Figure 39: Comparison of expression of *PGSC0003DMG402017989* putatively encoding cyclic nucleotide-gated ion channel 1-like protein in stage-1, stage-2, stage-3 and stage-4 samples of 6 cultivars using real-time PCR method. Data presented in each bar are mean expression value of 3 cDNA samples collected from 3 different potato plants of the same cultivar (presented in appendix B). (The Ct values of gene expression presented in appendix D.3.3).

4.3.4 Expression of gene *PGSC0003DMG400022929*, putatively encoding aminotransferase ALD1

Overall, this gene performed consistent expression patterns in both RNA sequencing method and real-time PCR method. RNA sequencing data performed the unique expression of this gene in stage-1 infected samples of cultivar Saturna and no expression neither in non-infected samples nor in infected samples of cultivar Beate (figure 40A). Similarly, real-time PCR data also described up-regulated expression of this gene in stage-1 infected samples of cultivar Saturna, very low expression of this gene in stage-1 non-infected samples of cultivar Saturna. Whereas the gene did not express in Beate, both in non-infected and infected samples (figure 40B).

Compared expression of this gene in 6 cultivars, real-time PCR data performed the highest expression pattern of this gene in stage-1 infected samples of cultivar Saturna, up to nearly 7 fold; and nearly 3 fold in stage-1 non-infected samples of cultivar DSxAs. Noticeably, this gene did not express in stage-1 non-infected and infected samples of cultivar Beate.

Similarly, the expression of this gene in stage-1 non-infected and infected samples of cultivar Tivoli were very low. In stage-2 samples, the gene expressed in all cultivars, including cultivar Beate and cultivar Tivoli (figure 41).

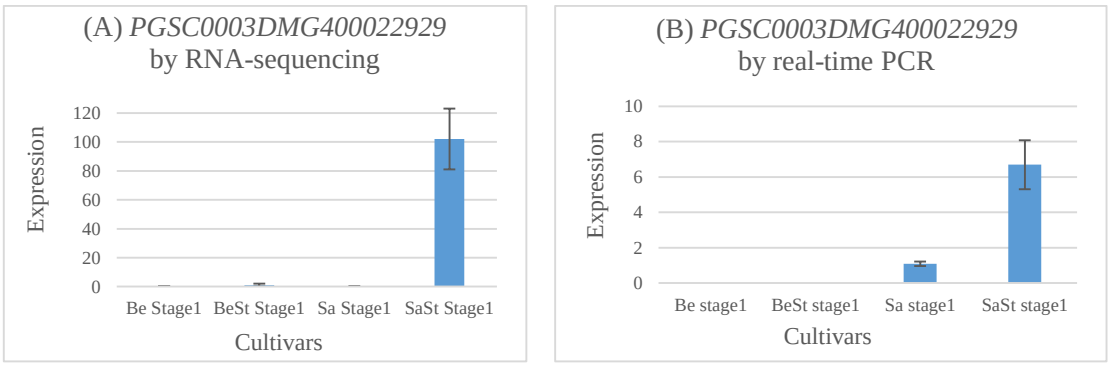


Figure 40: comparisons of expression of *PGSC0003DMG400022929*, putatively encoding aminotransferase ALD1, at stage-1 non-infected and infected samples of cultivar Beate and cultivar Saturna (A) using RNA-sequencing (RNA-sequencing data were supplied by NIBIO) and (B) using real-time PCR. RNA samples used in these methods were obtained from two independent experiments infected with the same *S. turgidiscabies* isolates.

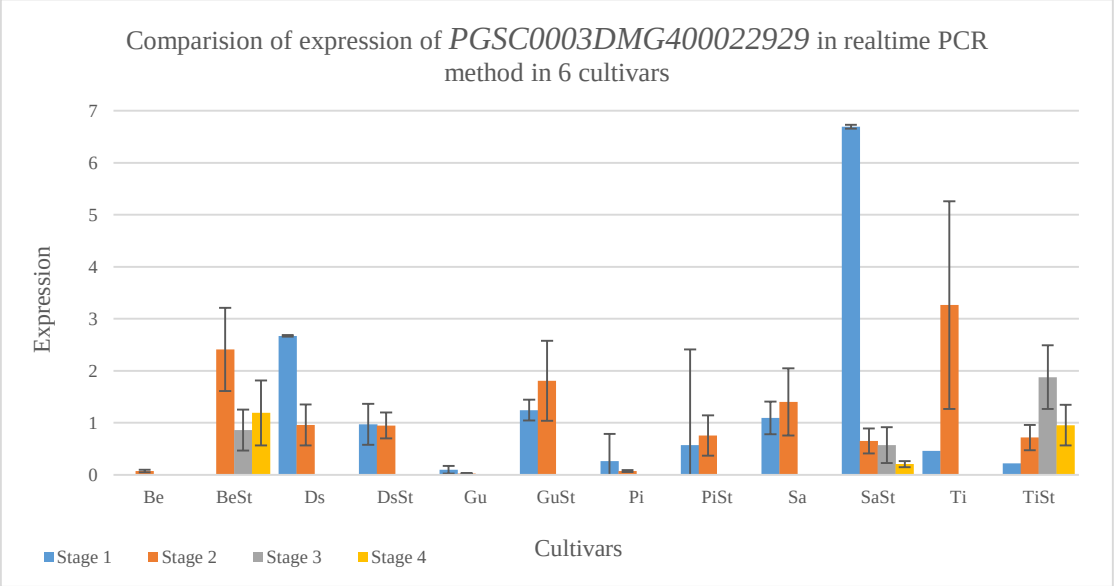


Figure 41: Comparison of expression of *PGSC0003DMG400022929*, putatively encoding aminotransferase ALD1, in stage-1, stage-2, stage-3 and stage-4 samples of 6 cultivars using real-time PCR method. Data presented in each bar are mean expression value of 3 cDNA samples collected from 3 different potato plants of the same cultivar (presented in appendix B). (The Ct values of gene expression presented in appendix D.4).

4.3.5 Expression of putative candidate auxin-associated genes

4.3.5.1 Expression of gene *PGSC0003DMG401008875,* with predicted function of ATP binding cassette transporter B family (ABCB)

Overall, this gene performed similar expression patterns in both RNA sequencing method and real-time PCR method. RNA sequencing data indicated higher expression of this gene in Beate and higher expression in infected samples of both Beate and Saturna (figure 14a). Similarly, real-time PCR data also described higher expression of this gene in Beate than expression of this gene in Saturna. However, the differences of expression in non-infected samples and infected samples of both cultivars were not significant (figure 42).

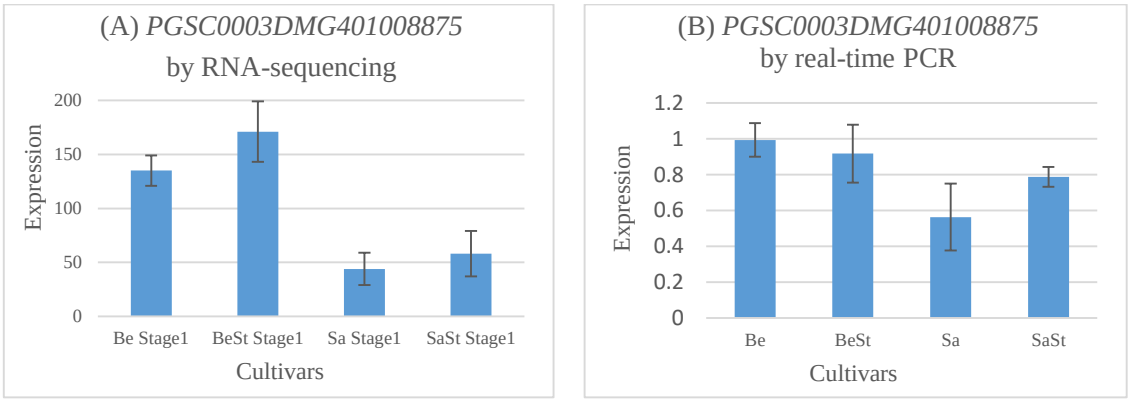


Figure 42 comparisons of expression of Gene *PGSC0003DMG401008875,* with predicted function of ATP binding cassette transporter B family (ABCB), in stage-1 non-infected and infected samples of cultivar Beate and cultivar Saturna (A) using RNA-sequencing (RNA-sequencing data were supplied by NIBIO) and (B) using real-time PCR. RNA samples used in these methods were obtained from two independent experiments infected with the same *S. turgidiscabies* isolates.

Real-time PCR data generally performed similar expression patterns in stage-1 samples of all cultivars, ranging from around 0.8 to 1.2 normalized folds; and higher expression patterns in stage-2 samples, ranging from around 1 to 2 fold, particularly up to around 5 fold in Tivoli non-infected samples (figure 43).

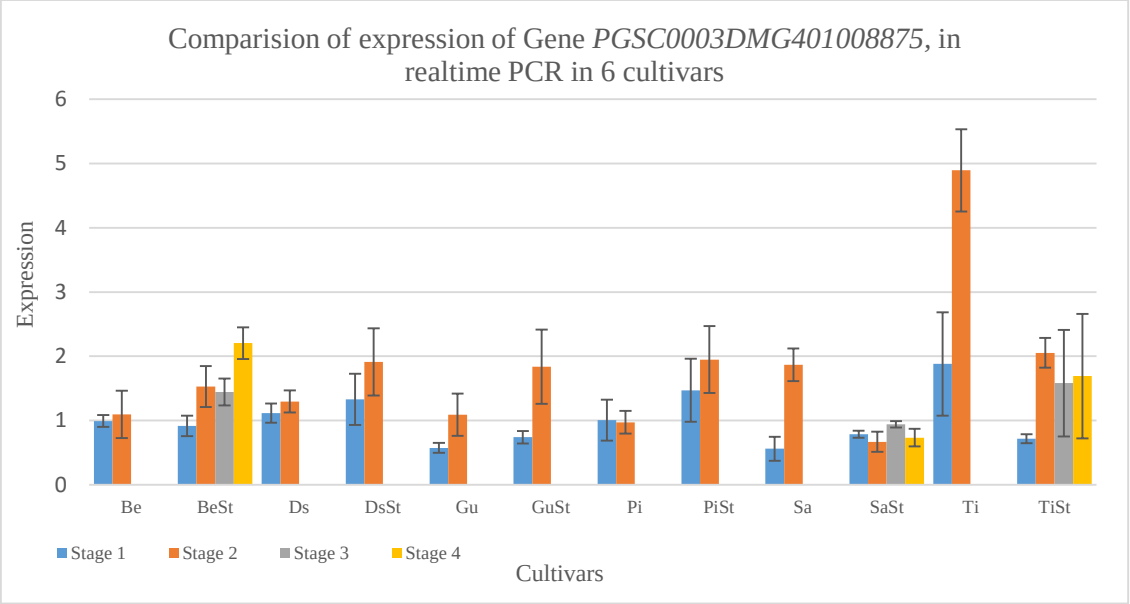


Figure 43: Comparison of expression of Gene *PGSC0003DMG401008875*, with predicted function of ATP binding cassette transporter B family (ABCB), in stage-1, stage-2, stage-3 and stage-4 samples of 6 cultivars using real-time PCR method. Data presented in each bar are mean expression value of 3 cDNA samples collected from 3 different potato plants of the same cultivar (presented in appendix B). (The Ct values of gene expression presented in appendix D.5.1).

4.3.5.2 Expression of gene *PGSC0003DMG401020044*, with predicted function of auxin binding protein 1 (ABP1)

Overall, this gene performed consistent expression patterns in both RNA-sequencing and realtime PCR. RNA-sequencing data indicated 2 alternative spliced transcripts of this gene. The accumulated expression pattern of this gene in RNA-sequencing described higher expression patterns in cultivar Saturna than those in cultivar Beate, in which, infected samples of both cultivars performed slightly higher expression than non-infected samples (figure 44A). Realtime PCR performed similar expression patterns with RNA-sequencing data in both cultivars (figure 44B).

Compared expression of this gene in 6 cultivars, real-time PCR data performed the highest expression pattern of this gene in stage-2 infected samples of cultivar Beate, up to around 4.7 fold. Overall expression in stage-1 and stage-2 samples, the gene performed highest expression in 2 resistant cultivar Beate and cultivar Tivoli. The lower expression patterns

were performed in susceptible cultivar DSxAs and cultivar Saturna. The lowest expression patterns were seen in the most susceptible cultivar Gullauge and cultivar Pimpernel (figure 45).

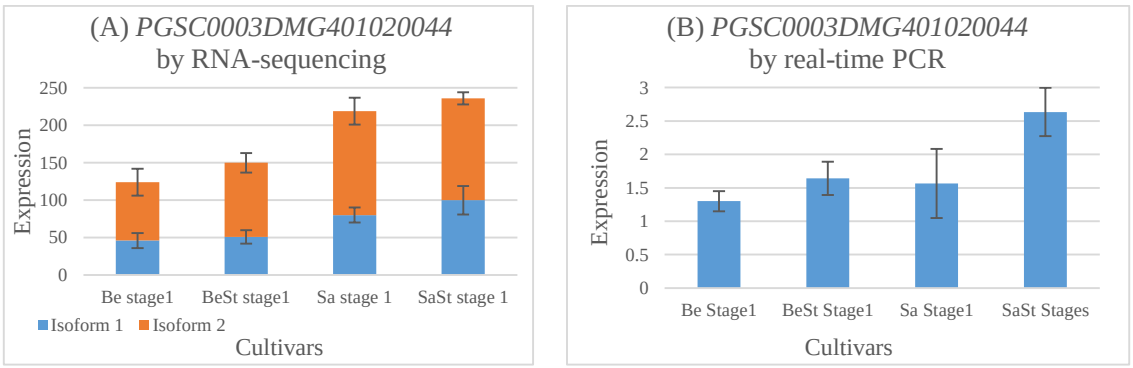


Figure 44: comparisons of expression of *PGSC0003DMG401020044*, with predicted function of auxin binding protein 1 (ABP1), in stage-1 non-infected and infected samples of cultivar Beate and cultivar Saturna (A) using RNA-sequencing (RNA-sequencing data were supplied by NIBIO) and (B) using real-time PCR. RNA samples used in these methods were obtained from two independent experiments infected with the same *S. turgidiscabies* isolates.

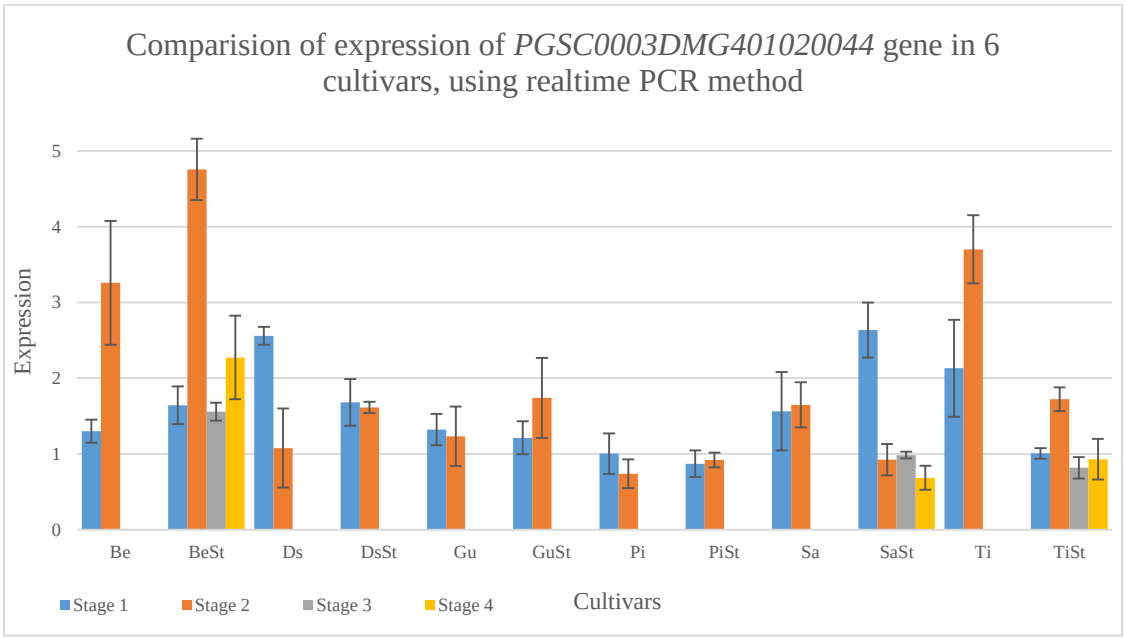


Figure 45: Comparison of expression of *PGSC0003DMG401020044*, with predicted function of auxin binding protein 1 (ABP1), in stage-1, stage-2, stage-3 and stage-4 samples of 6 cultivars using real-time PCR method. Data presented in each bar are mean expression value of 3 cDNA samples collected from 3 different potato plants of the same cultivar (presented in appendix B). (The Ct values of gene expression presented in appendix D.5.2).

5. DISCUSSIONS

5.1 Virulence of *Streptomyces turgidiscabies* isolates

The ability of producing melanin, a soluble brown pigment, has been used to characterize pathogenic streptomycetes (Thaxter 1891). As can be seen from figure 10, three OMA-plates inoculated with three different *S. turgidicabies* isolates turned brown, whereas the negative control plate still remained the original color of OMA medium (figure 6 and 10). This indicates that three *S. turgidicabies* isolates used for infecting radish seeds produced melanin as expected.

The ability of producing thaxtomin A has been used to characterize common scab-causing streptomycetes (Loria et al. 1997). The results from infection experiment on radish seeds described in section 4.1 shows that three *S. turgidicabies* isolates significantly inhibited seed germination (table 7). In addition, these three *S. turgidicabies* isolates also cause disease symptoms as expected, including stunting seedlings without roots, necrosis on leaves and hypertrophy (figure 10). These symptoms are consistent with symptoms caused by thaxtomin A on radish as described by Loria et al. (1997) and Dees et al. (2013). Thus, the results obtained from infection experiment on radish indicate that three *S. turgidicabies* isolates used to infect radish seeds produced thaxtomin A as expected.

5.2 Symptom on potato plants

The lesions on the stolon brought about from the infection experiment on potato as shown in section 4.2 (figure 13) is the symptom that consistently occurred in all infected samples of all investigated cultivars. These lesions are similar to common scab symptom in potato described by Baang (1979), Loria et al. (1997) and Dees et al. (2015). Since the results from infection experiment on radish indicated that three investigated *S. turgidicabies* isolates produced thaxtomin A, it is likely that symptom described in figure 13 was caused by these three thaxtomin A-producing *S. turgidicabies* isolates. The percentages of lesion area presented in table 8 indicate that cultivar Beate is absolutely resistant to these thaxtomin A-producing *S. turgidicabies* isolates (0% lesion area). Likewise, cultivar Tivoli (with 8% lesion area) and cultivar Hårek (with 9% lesion area), are relative resistant to these *S. turgidicabies* isolates. Cultivar Saturna (with 14% lesion area) and cultivar DSxAs (with

15% lesion area) are more susceptible. Whereas, cultivar Pimpernel (with 29% lesion area) and cultivar Gullauge (with 24% lesion area) are most susceptible. These results are as predicted and relatively consistent with resistant scores indicated by Møllerhagen (2014) (table 1). Therefore, lesions brought about from the infection experiment on potato as shown in figure 13 are considered to be symptom of common scab disease caused by *S. turgidicabies*.

However, similar symptom was also recorded on stolons and tubers of non-infected potato plants for negative control with lower percentages of lesion area than those in infected plants (table 8). In this experiment, although infected plants and negative-control plants were placed on 2 separate tables, both tables shared the same drip irrigation system (figure 8), and the system was mistakenly set up in the wrong direction with water flowing from infected plants to negative-control plants. Therefore, it is likely that spores were disseminated throughout the irrigation system, hence infected negative-control plants. Since 2 plates of YME medium were added into each pot of negative-control plant as described in section 3.2.2, YME medium may promote the growth of *Streptomyces* inoculum and make it sufficient to cause common scab symptom. The percentages of lesion area in infected plants are higher than those in non-infected plants, this is consistent with a finding from a previous work referring a correlation between pathogenic *Streptomyces* density in the root zone and disease incidence (Wang & Lazarovits 2005). In this experiment, growth-medium was supposed to be clean. Seed tubers were not neither treated before grown nor obtained *in vitro* because it was recently suggested that the ability of seed tuber transmitting common scab disease caused by *S. turgidiscabies* and *S. europaeiscabiei* is insignificant (Johansen et al. 2015). Taken together, the symptom occurred in negative-control plants is likely to be caused by *S. turgidiscabies* isolates transferred from infected pots to non-infected pots via the irrigation system.

To avoid unexpected infection in negative-control plants, two separate irrigation systems should be required for 2 tables. Seed tubers might need to be obtained *in-vitro* or treated with fludioxonil or biopesticide containing *Bacillus subtilis* (Al-Mughrabi et al. 2016). Additionally, growth-medium should be sterilized as refined in pathogenicity assay

conducted by Dees et al. (2015) or should be fumigated with Pic-Plus or chloropicrin (Al-Mughrabi et al. 2016).

5.3 Quantitative expressions of reference genes for normalization

Three standard curves obtained for quantitative expression of three reference genes in cDNA templates ranging from 0.03ng to 300ng have excellent correlation coefficient values of above 0.997, good amplification efficiencies within the range of 90% to 110%, and good slopes within the range of -3.58 and -3.10. All Ct values obtained in the real-time PCR are within the range of standard curves (figure 14, 16, 18). The melting curves of quantitative expression of three reference genes identify only 1 melt peak for each gene (figure 15A, 17A, 19A). All of these data indicate that the reactions were successful and primers are specific. However, different pictures of the quantitative expression of three reference genes in four stages of tuber development in 6 cultivars emerged.

The dissociation curve analyses for expression of gene *PGSC0003DMG400003985,* putatively encoding actin, show perfect pictures as expected, with unique melt curve at the same temperature in samples at four stages of tuber development. Melt curve also determines similar changes in relative fluorescence units with time (figure 15B, C, D). These data are consistent with corresponding Ct values (appendix D.1.1).This indicates that the expression of the gene is stable in four stages of tuber development in 6 investigated cultivars.

Differently, the dissociation curve analyses for expression of other two reference genes show expression data likely to be influenced by several errors. The dissociation curve analyses for expression of gene *PGSC0003DMG400044276,* putatively encoding 18SrRNA, show one major melt peak at 88^{o}C and multiple melt peaks at lower temperature (figure 19B, C, D). These unexpected melt peaks might suggest primer-dimer artifact when primers anneal to themselves and form small templates for PCR amplification. Alternatively, unexpected melt peaks might suggest unspecific PCR products resulted from mispriming when primers annealing to non-target complementary sequences.

In the expression of gene *PGSC0003DMG400023270,* putatively encoding elongation factor 1-α, the dissociation curve analyses show specific PCR amplicons with one consistent melt curve at 81.5^{o}C throughout four stages of tuber development (figure 17B, C, D). However,

a significant variation in the change of relative fluorescence units with time is determined. The change of relative fluorescence units in stage-3 and stage-4 samples ranges from 10 to above 600 units (figure 17D). These data are consistent with corresponding Ct values ranging within 24.02 and 39.94 (appendix D.1.2). This indicates the different expression levels of the gene at stage-3 and stage- 4 of tuber development in different cultivars.

As all discussed above, only gene *PGSC0003DMG400003985,* putatively encoding actin, is reliable for real-time PCR normalization in this study.

5.4 The correlation between expression of candidate genes and the infection caused by *Streptomyces turgidiscabies*

Although mRNA samples used to analyze expression of candidate genes by RNA-sequencing and real-time PCR were achieved from two different *S. turgidiscabies*-infection experiments (infected with the same isolates), expression patterns of most candidate genes in early hook stage samples of cultivar Beate and cultivar Saturna are consistent in two methods (the comparisons are presented in section 4.3). These consistent expression patterns constitute a putative correlation between the expression of candidate genes and the infection caused by *S. turgidiscabies,* in which, candidate genes are supposed to express in response to *S. turgidiscabies*-infection. This correlation suggests that RNA-sequencing method employed in this on-going project is a very useful tool for candidate gene selection.

However, 3 out of 13 investigated candidate genes performed inconsistent expression patterns in two methods. These inconsistent expression patterns might suggest a variation in alternative spliced transcripts, such as gene *PGSC0003DMG400000655,* putatively coding serine threonine-protein phosphatase-7, performed expression of 4 isoforms (figure 30A). Alternatively, functions of most candidate genes related to defense response were suggested in *Arabidopsis thaliana.* Thus, they might not have the same functions in potato and therefore their expressions might not be related to infection or defense response. For instance, gene *PGSC0003DMG400013405,* putatively encoding *myb*-related protein myb4 (figure 28A, B), was suggested to have a function in plant defense upon pathogen attack in *A. thaliana* (Van Verk et al. 2009). Whereas, *myb*-related protein were discussed to be involved in a diversity of functions, of those, myb4 protein in rice was found to play a role

in seed maturation (Suzuki et al. 1997). As all discussed above, the inconsistent expression patterns of 3 candidate genes in two methods might suggest some ideas for more successful candidate gene selection, that are isoform should not be selected for candidate gene because alternative splicing is unpredictable and different alternative spliced transcripts of the same gene may have different functions (Lesk 2012) and functions of candidate genes should be more specific for a putative common scab resistance strategy.

5.5 The correlation between expression of candidate genes and common scab resistance

The results from the real-time PCR expression of all 13 candidate genes can be interpreted in a way that suggests expressions of most candidate genes are not correlated to common scab resistance, including seven candidate genes uniquely expressed in resistant cultivar Beate. Only expressions of two candidate genes determined putative correlations to common scab resistance.

5.5.1 The correlation between expression of putative candidate defense-associated genes uniquely expressed in resistant cultivar Beate and common scab resistance

As presented in section 4.3.2, the results from real-time PCR indicate that seven candidate genes, which uniquely expressed in resistant cultivar Beate using RNA-sequencing analysis, were up-regulated in cultivar Beate as expected. However, expression of these 7 candidate genes in resistant cultivar Tivoli performed in unexpected ways, of which, three genes did not express in cultivar Tivoli at four stages of tuber development (figure 25, 27, and 31), while the presences of these genes in the genome were detected by real-time PCR in all cultivars (appendix D.6). The other 3 candidate genes expressed at very low levels in cultivar Tivoli in compare to those in cultivar Beate at all stages of tuber development (figure 21, 23, and 33). Noticeably, 1 candidate gene was up-regulated in susceptible cultivar Saturna with highest expression level among 6 investigated cultivars (figure 29). It is conceivable that no correlation between expression of these 7 candidate genes and common scab resistance was indicated as hypothesized.

Probably, a lack of correlation between the expressions of these 7 candidate genes and common scab resistance might be because of unspecific defense response to *Streptomyces*

infection. For instance, gene *PGSC0003DMG400000655* , putatively coding serine threonine-protein phosphatase-7 (figure 31), was suggested involving in response to light and stress signaling (Andreeva & Kutuzov 2009). It is likely that this function is not specific for common scab resistance, therefore, no correlation between its expression and common scab resistance is conceivable. On the other hand, it was found in *Arabidopsis* that 70% of genes related to defense regulation, which were up-regulated in thaxtomin A treatment, were also up-regulated in response to ozone treatment, wounding, bacterial elicitors and H_2O_2 treatment (Duval & Beaudoin 2009). This might implicate that the unique expressions of 7 candidate genes in cultivar Beate might be just in response to thaxtomin A, or cell wall wounded by thaxtomin A, or bacterial elicitors. The expressions were not specifically related to common scab resistance. Another explanation for the lack of correlation is probably because these candidate genes might not be required for common scab resistance since 4 of them putatively encode disease resistance proteins. Disease resistance proteins are known usually accumulated in vacuole (Taiz & Zeiger 2010; Yamada et al. 1999) and discharged into apoplast to suppress bacterial pathogen by proteasome-mediated membrane fusion, in which, plasma membrane and tonoplast are fused together (Hara-Nishimura & Hatsugai 2011). In case of *Streptomyces* infection, cell wall was indicated as thaxtomin A's target (Fry & Loria 2002) and thaxtomin A was microscopically observed to cause cell wall thickening and cell wall detached from plasmalemma in potato (figure 2B) (Goyer et al. 2000). Therefore, it might be impossible for proteasome-mediated membrane fusion, hence, disease resistance protein might not be required for common scab resistance.

These unexpected results are not in the agreement with previous study pronounced that the putative candidate defense-correlated genes uniquely expressed in cultivar Beate might involve in common scab resistance (Dees et al. 2015). As all discussed above, the lack of correlation between expressions of 7 putative candidate defense-associated genes uniquely expressed in resistant cultivar Beate and common scab resistance is prone to selecting candidate genes regardless of putative common scab-resistance strategy.

5.5.2 The correlation between expression of putative thaxtomin A-associated candidate genes and common scab resistance

As presented in section 4.3.3, the results from real-time PCR overall indicate that two genes, putatively encoding TXR1 proteins with function of thaxtomin A transport, similarly expressed in all cultivars regardless of non-infected and infected samples, and regardless of resistance and susceptibility to common scab (figure 35, and 37). This indicates that there is no correlation between the expression of these genes and common scab resistance. Although the function of *TXR1* gene were characterized as TXR1 protein in *Arabidopsis,* homologs of *TXR1* gene have been found in many plants (Scheible et al. 2003), including potato (Bombarely et al. 2011). Supposedly, gene *PGSC0003DMG400014859* and gene *PGSC0003DMG400025578* have function of thaxtomin A transport in potato, a lack of correlation between expression of these genes and common scab resistance implicates that thaxtomin A might be similarly transported within the cells in all cultivars. This implication conceives of a question whether thaxtomin A was similarly produced in resistant cultivars and susceptible cultivars or not. There might be some differences in thaxtomin A productions between resistant cultivars and susceptible ones. Since thaxtomin A is only produced in living host tissues and required cellobiose and cellotriose to initiate the biosynthesis pathway (Francis et al. 2015; Johnson et al. 2007), some differences in cellobiose and cellotriose synthesis in resistant cultivars and susceptible cultivars should be hypothesized. Another explanation for the lack of correlation between expression of these genes and common scab resistance might be some differences in ability of thaxtomin A-detoxification between resistant cultivars and susceptible ones (Acuña et al. 2001; King et al. 2000).

Nevertheless, transcription is just the first step in gene expression (Lesk 2012). The lack of correlation between expression of putative candidate thaxtomin A-associated genes and common scab resistance might also suggest some differences in translations of these genes in resistant cultivars and susceptible cultivars. Since an analysis of translation of these genes in 6 investigated cultivars was not carried out, it is still unknown whether these genes were similarly translated in resistant cultivars and susceptible cultivars or not. Alternatively, differences in lengths and sequences of the genes in different cultivars might lead to different functions but not lead to different transcriptions. For instance, wild-type *TXR1* gene in thaxtomin A-susceptible *Arabidopsis,* with stop codon at amino acid 116 and mutated *txr1*

gene in thaxtomin A-resistant *Arabidopsis*, with stop codon at amino acid 98, were found to perform similar transcriptions (Scheible et al. 2003). Therefore, DNA sequencing analysis of these genes in different cultivars might achieve some interesting answers.

5.5.3 The correlation between expression of putative candidate auxin-associated genes and common scab resistance

The expression of gene *PGSC0003DMG401020044*, with predicted function of auxin binding protein 1 (ABP1), noticeably determined a correlation between expression of this gene and common scab resistance as expected. Generally, the expression levels of the gene are likely to follow the order of resistance scores presented in table 8. The highest expression of the gene was seen in resistant cultivar Beate, and the lowest expression of the gene was observed in the most susceptible cultivar Pimpernel (figure 45). These results putatively indicate that ABP1 might involve in common scab resistance via auxin signaling pathway (as demonstrated in figure 4, section 1.7.3.2), in which, ABP1 was hypothesized to play a role in binding auxin, AUX/IAA repressor protein and auxin receptor together (Taiz & Zeiger 2010; Tromas et al. 2009). Conceivably, the higher expression levels of ABP1 putatively results in higher expression levels of gene *GH3*, contributing to auxin conjugation activities, cell wall strengthening and consequently disease resistance (as presented in section 1.7.3.2). Therefore, the highest expression of putative gene coding for ABP1 in cultivar Beate putatively indicates that cell wall strengthening might be a potential strategy to enhance common scab resistance in cultivar Beate. However, in cultivar Tivoli, the expression of the gene performed in an unexpected way, with higher expression patterns in non-infected samples than those in infected samples. An explanation for this is AUX/IAA repressor protein, a part of auxin receptor, might bind to the promotors of their own genes and suppress their own expression. In this case, ABP1 would not be required (Taiz & Zeiger 2010; Tiwari et al. 2003). Taken together, these expression patterns of gene *PGSC0003DMG401020044* might be conceived of a hypothesis that auxin signaling might promote common scab resistance in potato. This hypothesis is consistent with a suggestion from previous work that exogenous application of auxin via foliar spray promotes common scab resistance in potato as indicated by Tegg et al. (2008). However, auxin signaling depends on auxin level in the cell which is related to auxin transporter proteins and amino acids for auxin conjugation (as presented in section 1.7.3.2).

ATP binding cassette transporter B family (ABCB) was also considered to be putatively involved in auxin signaling pathway (Leyser 2006; Taiz & Zeiger 2010). The general picture emerging from the expression patterns of gene *PGSC0003DMG401008875*, putative coding for ABCB, is similar expression patterns in stage-1 and stage-2 samples of all cultivars and the gene was however not up-regulated in resistant cultivars as expected, only up-regulated in non-infected samples of cultivar Tivoli (figure 43). This draws a lack of correlation between the expression of this gene and common scab resistance. This putatively implicates that ABCB might not involve in auxin signaling pathway as predicted. Since the function of ABCB as regulating cellular auxin homeostasis has been studied in *Arabidopsis* (Lin & Wang 2005; Taiz & Zeiger 2010), ABCB of potato might have different functions or the gene *PGSC0003DMG401008875* might not coding for this function, or there may be other factors also involving in auxin transport. For instance, the PIN family has also been identified as auxin transporter, regulating cellular auxin homeostasis in *Arabidopsis* (Gälweiler et al. 1998; Marchant et al. 2002; Taiz & Zeiger 2010). Recently, the PIN family has been investigated in potato, of which, two PIN genes, *StPIN3* and *StPIN4*, were determined with highest expressions in elongation stolon samples (Roumeliotis et al. 2013). These findings from previous work might suggest *StPIN3* and *StPIN4* involving in auxin signaling in potato.

5.5.4 The correlation between expression of gene *PGSC0003DMG400022929*, putatively encoding aminotransferase ALD1 and common scab resistance

Aminotransferase ALD1 was suggested to play a role in generation of amino acid homeostasis in the cell (Kachroo & Robin 2013; Song et al. 2004) and in generation of an amino acid-derived defense signal (Song et al. 2004). Gene *PGSC0003DMG400022929* did not express in stage-1 non-infected and infected samples of resistant cultivar Beate and little expressed in stage-1 samples of resistant cultivar Tivoli. Whereas, the gene expressed in susceptible cultivars, particularly, it was most up-regulated in stage-1 samples of susceptible cultivar Saturna (figure 41). These expression patterns draw a correlation between expressions of the gene and common scab resistance. This correlation putatively indicates that amino acid homeostasis might be remained stable in very early stage of tuber development in resistant cultivar Beate and cultivar Tivoli, but not in susceptible cultivars. It is also putatively indicated that there was no amino acid-derived defense signal generated

in these two resistant cultivars in early hook stage of tuber development. These putative indications putatively conceive of an idea that cultivar Beate was not invaded and cultivar Tivoli was slightly invaded by *S. turgidiscabies* at initial stage of tuber development. Interestingly, percentages of lesion area obtained from the infection experiment on potato plants are also consistent with this hypothesis, 0% of lesion area for cultivar Beate and 8% of lesion area for cultivar Tivoli (table 8). Most recently, it was also suggested that resistance to *S. turgidiscabies* in potato might be achieved at initial stages of tuberization (Dees et al. 2015).

However, the gene expressed in stage-2 samples of cultivar Beate and cultivar Tivoli and no clear picture describing the expressions of the gene in stage-2 samples of all cultivars. It is likely that there was a perturbations in amino acid homeostasis in stage-2 samples in all cultivars, for instance, the gene was up-regulated in stage-1 infected samples of cultivar Saturna, up to about 6.6 fold, then, it was dramatically down-regulated in stage-2 infected samples, less than 1 fold. As discussed in section 5.5.3 that auxin signaling was hypothesized to promote common scab resistance in potato and auxin conjugation activity putatively occurred during auxin signaling pathway. Thus, the conjugation of auxin to amino acid might lead to some perturbations in amino acid homeostasis. Recently, it was suggested that an alterations in amino acid homeostasis might influence plant resistance to pathogen, and the perturbations in amino acid homeostasis may result in constitutive activation or suppression plant defenses (Zeier 2013). Unfortunately, the data obtained in this thesis are insufficient to constitute any further explanation or suggestion for the expressions of the gene in stage-2 samples in 6 investigating cultivars.

5.6 Concluding remarks

Infection experiment on radish seeds in a quick, easy and low-cost means to test for ability to produce thaxtomin A in common scab-causing *Streptomyces* species.

Infection experiment on potato plant indicated that cultivar Beate and cultivar Tivoli are resistant to common scab caused by *S. turgidicabies*.

Seven of candidate genes uniquely expressed in resistant cultivar Beate in RNA-sequencing did not indicate any correlation between their expression and common scab resistance in potato.

The expression patterns of gene *PGSC0003DMG401020044*, with predicted function of auxin binding protein 1 (ABP1), indicated a putative correlation with common scab resistance in potato. This correlation putatively conceived of an idea that auxin signaling might promote common scab resistance in potato.

The expression patterns of gene *PGSC0003DMG400022929*, putatively encoding aminotransferase ALD1, also partly indicated a correlation with common scab resistance in potato. This correlation putative conceived of an idea that resistant cultivars might not be invaded or slightly invaded by *S. turgidiscabies*.

Gene *PGSC0003DMG400014859* and gene *PGSC0003DMG400025578*, putatively coding for TXR1 proteins involving in thaxtomin A transport, did not determine any correlation between their expression patterns and common scab resistance in potato.

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APPENDIX A – RNA-sequencing data of candidate genes for common scab resistance

Potato gene name	NCBI NR-database	Cultivar Beate				Cultivar Saturna			
		Be1	BeSt1	Be3	BeSt3	Sa1	SaSt1	Sa3	SaSt3
PGSC0003DMG400040744	Late blight resistance	77 ± 9	117 ± 7	73 ± 28	62 ± 7	0 ± 0	0 ± 0	0 ± 0	0 ± 0
PGSC0003DMG400017087	Late blight resistance	17 ± 2	266 ± 43	50 ± 14	23 ± 2	0 ± 0	0 ± 0	0 ± 0	0 ± 0
PGSC0003DMG400007385	Disease resistance protein at427190	130 ± 14	146 ± 8	46 ± 7	42 ± 9	0 ± 0	0 ± 0	0 ± 0	0 ± 0
PGSC0003DMG400010612	Disease resistance protein rpp13	26 ± 5	51 ± 4	126 ± 51	8 ± 3	0 ± 0	0 ± 0	0 ± 0	0 ± 0
PGSC0003DMG400013405	Myb-related protein myb4	23 ± 6	77 ± 10	67 ± 40	41 ± 20	2 ± 2	0 ± 0	0 ± 0	1 ± 1
PGSC0003DMG400000655	Serine-threonine-protein	512 ± 64	884 ±79	662 ± 97	450 ± 44	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		103 ± 12	214 ± 25	158 ± 35	62 ± 7	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		70 ± 7	103 ± 2	124 ± 12	49 ± 8	0 ± 0	1 ± 0	0 ± 0	1 ± 1
		82 ± 13	132 ± 7	151 ± 22	48 ± 12	0 ± 0	2 ± 1	0 ± 0	1 ± 1
PGSC0003DMG400015425	Sre 1a protein	1569 ± 91	4012 ± 411	2071 ± 828	1192 ± 142	3175 ± 570	3577 ± 1 041	2451 ± 357	2292 ± 424
		2770 ± 319	6051 ± 519	4947 ± 1 613	1778 ± 181	7005 ± 1 054	9802 ± 2 960	8596 ± 629	7459 ± 2 206
		553 ± 76	1329 ± 98	1377 ± 510	374 ± 35	2 ± 1	5 ± 2	2 ± 1	3 ± 2
		2847 ± 307	6928 ± 752	3614 ± 1 159	2001 ± 224	5358 ± 1 125	7722 ± 1 292	4902 ± 223	4640 ± 1 367
		209 ± 23	393 ± 29	295 ± 60	117 ± 9	76 ± 19	138 ± 29	44 ± 10	23 ± 7
		211 ± 24	388 ± 19	315 ± 68	146 ± 14	480 ± 80	773 ± 191	348 ± 40	195 ± 49
PGSC0003DMG400014859	Txr1 protein	77 ± 9	94 ± 5	55 ± 10	79 ± 6	42 ± 6	63 ± 5	40 ± 2	33 ± 9
PGSC0003DMG400025578	Txr1 protein	160 ± 21	146 ± 15	106 ± 11	166 ± 14	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		227 ± 22	198 ± 8	134 ± 15	197 ± 13	318 ± 38	428 ± 75	274 ± 33	248 ± 55
PGSC0003DMG402017989	Cyclic nucleotide-gated ion channel 1-like protein (CNG)	2 ± 1	21 ± 4	9 ± 4	1 ± 0	10 ± 2	151 ± 33	5 ± 2	11 ± 4
		5 ± 1	105 ± 23	4 ± 2	9 ± 0	7 ± 3	203 ± 46	1 ± 1	12 ± 4
		9 ± 9	143 ± 33	31 ± 6	9 ± 5	20 ± 4	161 ± 41	11 ± 8	35 ± 13
		11 ± 1	105 ± 36	6 ± 4	5 ± 1	11 ± 11	459 ± 63	4 ± 4	12 ± 7
		4 ± 1	34 ± 6	10 ± 5	1 ± 0	8 ± 1	180 ± 46	4 ± 3	14 ± 5
		9 ± 3	153 ± 27	20 ± 7	14 ± 2	14 ± 2	289 ± 44	9 ± 2	24 ± 8
		6 ± 1	29 ± 3	4 ± 1	5 ± 1	38 ± 3	167 ± 32	12 ± 2	21 ± 5
PGSC0003DMG400022929	Aminotransferase ALD1	0 ± 0	1 ± 1	0 ± 0	0 ± 0	0 ± 0	102 ± 21	1 ± 1	0 ± 0
PGSC0003DMG401008875	ATP binding cassette (ABC)	135 ± 14	171 ± 28	135 ± 31	87 ± 8	44 ± 15	58 ± 21	46 ± 6	42 ± 12
PGSC0003DMG401020044	Auxin binding protein 1 (ABP1)	46 ± 10	51 ± 9	43 ± 9	80 ± 11	80 ± 10	100 ± 19	53 ± 13	57 ± 14
		78 ± 18	99 ± 13	67 ± 5	46 ± 8	139 ± 18	136 ± 8	117 ± 15	99 ± 35

Notes: **Be1**: Stage-1 non-infected sample of cultivar Beate. **BeSt1**: Stage-1 *S. tugidiscabies*-infected sample of cultivar Beate. **Be3**: Stage-3 non-infected sample of cultivar Beate. **BeSt3**: Stage-1 *S. tugidiscabies*-infected sample of cultivar Beate. **Sa1**: Stage-1 non-infected sample of cultivar Saturna. **SaSt1**: Stage-1 *S. tugidiscabies*-infected sample of cultivar Saturna. **Sa3**: Stage-3 non-infected sample of cultivar Saturna. **SaSt3**: Stage-1 *S. tugidiscabies*-infected sample of cultivar Saturna.

APPENDIX B - Concentrations and RIN values of RNA samples

B.1 Stage-1 samples

Sample	Pot	Descriptions	RIN	Concentration (ng/µl)	25s/18s Ratio
1.s1	1	Beate + Strep.	8.4	3,038	2.4
3.s1	3	Beate + Strep.	10	2,236	2.0
5.s1	5	Beate + Strep.	8.6	1,712	2.5
56.s1	56	Beate	10	2,749	2.1
57.s1	57	Beate	9.9	2,948	2.3
55.s1	55	Beate	10	1,124	2.1
7.s1	7	DSxAs + Strep.	9.6	2,778	2.2
9.s1	9	DSxAs + Strep.	9.9	2,378	2.4
11.s1	11	DSxAs + Strep.	8.6	1,185	2.3
61.s1	61	DSxAs	8.2	1,600	3.6
64.s1	64	DSxAs	7.9	1,254	4.8
66.s1	66	DSxAs	8	1,704	4.3
21.s1	21	Gullauge + Strep.	8.9	1,376	2.3
22.s1	22	Gullauge + Strep.	8.3	660	5.5
24.s1	24	Gullauge + Strep.	8.3	836	4.4
73.s1	73	Gullauge	9.3	1,498	1.9
74.s1	74	Gullauge	9.2	994	2.7
76.s1	76	Gullauge	7.1	3,293	1.8
37.s1	37	Pimpernel + Strep.	8.5	639	2.3
38.s1	38	Pimpernel + Strep.	9.1	562	2.4
41.s1	41	Pimpernel + Strep.	6.5	2,073	2.4
93.s1	93	Pimpernel	6.8	3,674	1.6
94.s1	94	Pimpernel	7	1,111	1.7
95.s1	95	Pimpernel	7.9	2,860	1.7
43.s1	43	Saturna + Strep.	7.1	950	2.4
47.s1	47	Saturna + Strep.	7.6	3,431	3.5
48.s1	48	Saturna + Strep.	7	2,478	6.6
100.s1	100	Saturna	6.8	933	2.2
101.s1	101	Saturna	10	1,040	2.3
102.s1	102	Saturna	9.8	972	2.6
49.s1	49	Tivoli + Strep.	9.3	460	2.5
51.s1	51	Tivoli + Strep.	10	884	2.2
52.s1	52	Tivoli + Strep.	7.8	2,300	3.6
53.s1	53	Tivoli	9.8	873	2.3
105.s1	105	Tivoli	6	833	2.3
106.s1	106	Tivoli	7.5	1,289	2.4

B.2 Stage-2 samples

Sample	Pot	Descriptions	RIN	Concentration (ng/µl)	25s/18s Ratio
1.s2	1	Beate + Strep	8.4	245	3.6
3.s2	3	Beate + Strep	9.5	1,049	2.8
5.s2	5	Beate + Strep	8.9	462	3.1
55.s2	55	Beate	6.1	1,475	3.2
57.s2	57	Beate	7.4	1,021	2.6
60.s2	60	Beate	8.9	648	2.8
7.s2	7	DSxAs + Strep	6.1	428	11.2
9.s2	9	DSxAs + Strep	7.6	932	5.9
11.s2	11	DSxAs + Strep	6.9	515	8.2
63.s2	63	DSxAs	9	1,621	2.5
65.s2	65	DSxAs	7.3	1,898	3.4
66.s2	66	DSxAs	8.7	1,058	2.5
21.s2	21	Gullauge + Strep	10	974	2
22.s2	22	Gullauge + Strep	8.3	862	3
24.s2	24	Gullauge + Strep	9.7	775	2.2
73.s2	73	Gullauge	8.7	1,600	3
74.s2	74	Gullauge	7.3	2,205	3.4
76.s2	76	Gullauge	9	1,193	2.5
37.s2	37	Pimpernel + Strep	10	486	2.2
38.s2	38	Pimpernel + Strep	9.2	331	2.8
39.s2	39	Pimpernel + Strep	10	493	4.8
93.s2	93	Pimpernel	9	502	2.7
94.s2	94	Pimpernel	10	404	2.2
95.s2	95	Pimpernel	10	1,007	2.3
43.s2	43	Saturna + Strep	9.9	1,360	2.5
46.s2	46	Saturna + Strep	8.7	1,488	2.7
48.s2	48	Saturna + Strep	10	1,296	2.4
100.s2	100	Saturna	10	818	2.2
101.s2	101	Saturna	10	1,261	2.3
102.s2	102	Saturna	9.1	1,830	2.2
50.s2	50	Tivoli + Strep	10	1,427	2.2
52.s2	52	Tivoli + Strep	10	2,341	2.3
53.s2	53	Tivoli + Strep	10	1,387	2.4
100.s2	100	Tivoli	10	776	2.6
101.s2	101	Tivoli	10	1,087	2.5
101.s2	102	Tivoli	10	2,432	2.1

B.3 Stage-3 samples

Sample	Pot	Descriptions	RIN	Concentration (ng/µl)	25s/18s Ratio
4.s3	4	Beate + Strep	10	2,077	2.0
6.s3	6	Beate + Strep	10	1,614	2.0
58.s3	58	Beate	10	1,101	2.1
46.s3	46	Saturna + Strep	10	2,369	2.0
47.s3	47	Saturna + Strep	10	3,617	1.9
48.s3	48	Saturna + Strep	10	2,256	2.1
50.s3	50	Tivoli + Strep	10	1,796	2.4
51.s3	51	Tivoli + Strep	9.8	1,856	1.9
106.s3	106	Tivoli	9.6	595	2.2

B.4 Stage-4 samples

Sample	Pot	Descriptions	RIN	Concentration (ng/µl)	25s/18s Ratio
1.s4	1	Beate + Strep	10	1,285	1.9
55.s4	55	Beate	9.5	409	2.2
56.s4	56	Beate	9.1	521	2.3
43.s4	43	Saturna + Strep	10	1,660	2.0
46.s4	46	Saturna + Strep	10	2,891	2.0
47.s4	47	Saturna + Strep	9.5	1,634	2.3
49.s4	49	Tivoli + Strep	10	2,188	2.1
50.s4	50	Tivoli + Strep	10	2,311	2.0
106.s4	106	Tivoli	10	1,184	2.0

APPENDIX C – Concentrations, 260/280 ratio of DNA samples

Samples	Pot	Descriptions	Concentration (ng/µl)	260/280 ratio
1.s2	1	Beate + Strep	15.9	1.82
57.s2	57	Beate	8.6	2.18
60.s2	60	Beate	4.5	2.29
7.s3	7	DSxAs + Strep	6.9	2.45
65.s3	65	DSxAs	6.3	2.2
66.s3	66	DSxAs	13.0	1.67
21.s2	21	Gullauge + Strep	6.2	2.4
73.s2	73	Gullauge	16.7	2.03
74.s2	74	Gullauge	35.7	1.98
93.s2	93	Pimpernel	15.1	2.08
94.s2	94	Pimpernel	14.4	1.96
95.s2	95	Pimpernel	15.0	1.95
46.s2	46	Saturna + Strep	20.6	1.78
97.s5	97	Saturna	27.4	1.84
102.s2	102	Saturna	24.4	1.93
50.s2	50	Tivoli + Strep	18.2	1.98
53.s2	53	Tivoli + Strep	13.6	1.84
105.s5	105	Tivoli	32.0	1.71

APPENDIX D – Threshold Cycle values of gene expression

D.1 Internal reference genes for normalization

D.1.1 *PGSC0003DMG400003985* gene, putatively encoding actin

	Ct values	
Stage-1 Samples	Replicate 1	Replicate 2
Be55-1	19.35	19.24
Be56-1	20.91	20.89
Be57-1	20.27	20.12
BeSt1-1	21.84	22
BeSt3-1	19.77	19.86
BeSt5-1	20.76	20.72
Ds61-1	20.88	20.59
Ds64-1	21.09	21.18
Ds66-1	21.13	21.09
DsSt11-1	20.16	20.23
DsSt7-1	19.64	19.7
DsSt9-1	19.27	19.18
Gu73-1	18.11	18.14
Gu74-1	18.4	18.68
Gu76-1	19.34	19.4
GuSt21-1	18.96	19.11
GuSt22-1	19.97	20.02
GuSt24-1	20.82	20.92
Pi93-1	19.07	18.97
Pi94-1	20.03	19.98
Pi95-1	18.51	18.57
PiSt37-1	20.16	20.26
PiSt38-1	20.12	20.05
PiSt41-1	19.36	19.61
Sa100-1	23.35	23.17
Sa101-1	17.93	18.05
Sa102-1	18.61	18.48
SaSt43-1	21.01	21.2
SaSt47-1	21.67	21.69
SaSt48-1	22.37	22.28
Ti105-1	18.05	18.33
Ti106-1	19.21	19.21
TiSt49-1	19.3	19.36
TiSt51-1	19.23	19.27
TiSt52-1	19.63	19.56
TiSt53-1	19.53	19.74

	Ct values	
Stage-2 Samples	Replicate 1	Replicate 2
Be55-2	20.46	20.87
Be57-2	20.1	20.32
Be60-2	22.06	22.35
BeSt1-2	21.2	22.37
BeSt3-2	21.07	22.19
BeSt5-2	20.92	21.22
Ds63-2	21.63	21.58
Ds65-2	21.18	21.24
Ds66-2	22.66	22.67
DsSt11-2	22.57	22.64
DsSt7-2	23.61	24.05
DsSt9-2	21.04	21.52
Gu73-2	17.98	19.94
Gu74-2	19.11	21.17
Gu76-2	18.8	20.38
GuSt21-2	18.55	21.01
GuSt22-2	20.77	22.8
GuSt24-2	19.29	21.39
Pi93-2	18.63	19.63
Pi94-2	18.27	18.85
Pi95-2	17.65	18.14
PiSt37-2	21.67	22.33
PiSt38-2	20.65	21.56
PiSt39-2	22.13	22.26
Sa100-2	26.72	27.29
Sa101-2	20.35	20.35
Sa102-2	21.15	21.18
SaSt43-2	20.03	20.48
SaSt46-2	19.38	19.62
SaSt48-2	20.3	20.41
Ti104-2	20.56	20.94
Ti105-2	19.75	20
Ti106-2	20.21	20.22
TiSt50-2	19.55	19.81
TiSt52-2	19.6	19.95
TiSt53-2	20.31	20.54

Stage-3 Samples	Replicate 1	Replicate 2
Be3-4	20.19	20.32
Be3-58	20.29	20.03
Be3-6	20.24	20.03
Sa3-46	19.84	19.67
Sa3-47	19.27	19.07
Sa3-48	19.03	19.21
Ti3-106	22.05	22.12
Ti3-50	20.62	20.85
Ti3-51	21.03	20.63

Stage-4 Samples	Replicate 1	Replicate 2
Be4-1	20.19	20.55
Be4-55	22.88	22.97
Be4-56	22.7	22.78
Sa4-43	21.36	21.89
Sa4-46	19.52	19.48
Sa4-47	19.47	19.74
Ti4-106	21.86	22.24
Ti4-49	19.62	19.85
Ti4-50	19.64	19.48

D.1.2 *PGSC0003DMG400023270* gene, putatively encoding elongation factor1-α

	Ct values	
Stage-1 Samples	Replicate 1	Replicate 2
Be55-1	20.7	19.74
Be56-1	20.62	20.97
Be57-1	20.62	19.4
BeSt1-1	20.56	20.97
BeSt3-1	19.82	19.92
BeSt5-1	20.26	20.1
Ds61-1	27.96	27.98
Ds64-1	28.07	27.86
Ds66-1	28.66	28.47
DsSt11-1	27.83	27.97
DsSt7-1	28.22	28.56
DsSt9-1	27.08	27.2
Gu73-1	19.25	20.02
Gu74-1	19.6	20.02
Gu76-1	21.2	21.27
GuSt21-1	20.93	21.08
GuSt22-1	20.97	20.66
GuSt24-1	21.34	21.43
Pi93-1	20.08	20.26
Pi94-1	20.27	20.7
Pi95-1	20.5	20.35
PiSt37-1	19.75	20.53
PiSt38-1	20.24	20.58
PiSt41-1	20.44	20.37
Sa100-1	20.41	20.62
Sa101-1	17.84	17.98
Sa102-1	17.57	17.82
SaSt43-1	18.2	18.94
SaSt47-1	19.67	20.41
SaSt48-1	19.93	20.66
Ti105-1	17.62	18.42
Ti106-1	18.12	20.28
TiSt49-1	19.09	20.2
TiSt51-1	18.17	19.07
TiSt52-1	19.04	20.23
TiSt53-1	18.03	18.29

	Ct values	
Stage-2 Samples	Replicate 1	Replicate 2
Be55-2	19.05	19.33
Be57-2	18.86	19.43
Be60-2	19.48	20.27
BeSt1-2	19.51	20.45
BeSt3-2	19.94	20.12
BeSt5-2	19.04	19.65
Ds63-2	22.73	22.38
Ds65-2	22.3	22.27
Ds66-2	23.21	23.03
DsSt11-2	23.23	22.76
DsSt7-2	23.84	24
DsSt9-2	22.71	22.75
Gu73-2	16.97	16.6
Gu74-2	18.26	18.23
Gu76-2	18.17	16.69
GuSt21-2	16.71	18.3
GuSt22-2	19.06	19.14
GuSt24-2	16.77	17.12
Pi93-2	16.75	17.18
Pi94-2	17.01	17.18
Pi95-2	17.28	16.04
PiSt37-2	17.71	18.01
PiSt38-2	18.13	18.22
PiSt39-2	19.41	19.82
Sa100-2	22.31	22.5
Sa101-2	16.57	17.03
Sa102-2	16.71	17.79
SaSt43-2	17.18	17.5
SaSt46-2	16.87	17.01
SaSt48-2	18.6	18.66
Ti104-2	19.22	19.17
Ti105-2	18.89	18.95
Ti106-2	18.42	17.44
TiSt50-2	18.12	18.17
TiSt52-2	19.22	19.2
TiSt53-2	18.87	18.53

Stage-3 Samples	Replicate 1	Replicate 2
Be3-4	39.94	39.22
Be3-58	35.36	N/A
Be3-6	26.23	29.73
Sa3-46	31.6	31.16
Sa3-47	36.93	34.83
Sa3-48	31.63	29.33
Ti3-106	31.33	33.01
Ti3-50	N/A	38.13
Ti3-51	24.02	24.76

Stage-4 Samples	Replicate 1	Replicate 2
Be4-1	36.32	35.3
Be4-55	28.95	29.39
Be4-56	24.3	23.96
Sa4-43	33.04	35.35
Sa4-46	25.4	25.57
Sa4-47	27.07	24.36
Ti4-106	29.35	33.83
Ti4-49	30.46	28.06
Ti4-50	25.05	27.26

D.1.3 *PGSC0003DMG400044276* gene, putatively encoding 18SrRNA

	Ct values	
Stage-1 Samples	Replicate 1	Replicate 2
Be55-1	11.04	11.75
Be56-1	11.13	11.50
Be57-1	10.93	11.99
BeSt1-1	12.18	13.40
BeSt3-1	10.82	12.13
BeSt5-1	12.04	12.09
Ds61-1	12.35	12.15
DsSt64-1	13.06	13.23
DsSt66-1	13.39	13.92
DsSt7-1	12.15	11.58
DsSt9-1	12.30	13.19
DsSt11-1	12.92	13.30
Gu73-1	14.96	13.33
Gu74-1	13.22	13.84
Gu76-1	12.10	12.21
GuSt21-1	13.30	13.22
GuSt22-1	17.97	16.40
GuSt24-1	22.99	21.50
Pi93-1	14.38	12.73
Pi94-1	16.63	12.53
Pi95-1	13.87	10.89
PiSt37-1	35.02	14.66
PiSt38-1	15.20	13.57
PiSt41-1	14.26	13.51
Sa100-1	16.66	14.85
Sa101-1	13.97	14.18
Sa102-1	12.91	12.71
SaSt43-1	17.62	16.13
SaSt47-1	13.62	13.67
SaSt48-1	12.51	12.78
Ti105-1	15.48	15.00
Ti106-1	15.93	15.60
TiSt53-1	19.33	18.38
TiSt49-1	14.95	17.38
Tist51-1	22.12	18.73
TiSt52-1	16.04	15.12

	Ct values	
Stage-2 Samples	Replicate 1	Replicate 2
Be55-2	15.94	17.75
Be57-2	21.71	22.17
Be60-2	24.06	23.08
BeSt1-2	20.12	21.24
BeSt3-2	18.59	19.5
BeSt5-2	21.98	22.8
Ds63-2	22.28	20.59
Ds65-2	16.95	17.1
Ds66-2	23.29	23.17
DsSt11-2	25.97	26.16
DsSt7-2	24.25	24.88
DsSt9-2	23.08	23.67
Gu73-2	19.48	21.21
Gu74-2	19.87	20.62
Gu76-2	25.28	24.64
GuSt21-2	23.14	23.06
GuSt22-2	24.05	25.37
GuSt24-2	19.26	20.18
Pi93-2	27.76	30.09
Pi94-2	25.82	27.08
Pi95-2	22.43	28.13
PiSt37-2	29.83	33.68
PiSt38-2	29.47	30.56
PiSt39-2	26.5	28.28
Sa100-2	37.35	37.31
Sa101-2	34.31	31.08
Sa102-2	30.7	30.7
SaSt43-2	22.35	25.13
SaSt46-2	22.18	25.98
SaSt48-2	19.24	23.3
Ti104-2	21.37	19.53
Ti105-2	20.1	19.86
Ti105-2	22.42	23.87
TiSt50-2	23.18	16.83
TiSt52-2	17.44	20.56
TiSt53-2	23.5	20.68

Stage-3 Samples	Replicate 1	Replicate 2
Be3-4	28.72	20.3
Be3-58	13.61	14.56
Be3-6	14.71	14.97
Sa3-46	18.35	18.57
Sa3-46	15.19	15.4
Sa3-47	14.07	19.47
Ti3-50	15.40	18.57
Ti3-51	13.36	12.98
Ti3-106	17.32	18.03

Stage-4 Samples	Replicate 1	Replicate 2
Be4-1	13.14	17.38
Be4-55	15.98	17.01
Be4-56	14.94	14.03
Sa4-43	19.54	17.99
Sa4-46	13.68	14.66
Sa4-47	15.12	15.22
Ti4-106	19.15	19.39
Ti4-49	13.41	15.7
Ti4-50	13.97	13.71

D.2 Genes uniquely expressed in the resistant cultivar Beate

D.2.1 Gene *PGSC0003DMG400040744*, putatively coding for late blight resistance protein

	Ct values	
Stage-1 Samples	Replicate 1	Replicate 2
Be55-1	27.18	26.87
Be56-1	27.36	27.84
Be57-1	26.58	27.07
BeSt1-1	27.33	27.72
BeSt3-1	26.5	26.9
BeSt7-1	27.13	27.53
Ds61-1	27.54	28.69
Ds64-1	27.7	28.63
Ds66-1	28.41	29.46
DsSt11-1	26.65	27.39
DsSt7-1	27.99	28.94
DsSt9-1	27.57	28.87
Gu73-1	28.14	27.47
Gu74-1	27.94	27.23
Gu76-1	28.56	28.04
GuSt21-1	29.6	28.07
GuSt22-1	29.99	29.3
GuSt24-1	30.14	29.23
Pi93-1	32.54	28.36
Pi94-1	31.32	29.14
Pi95-1	28.46	28.27
PiSt37-1	30.05	30.32
PiSt38-1	30.06	30.13
PiSt41-1	29.03	28.95
Sa100-1	29.52	30.11
Sa101-1	29.47	30.03
Sa102-1	30.11	30.48
SaSt43-1	28.69	28.91
SaSt47-1	29.84	29.91
SaSt48-1	27.12	27.43
Ti105-1	28.01	28.38
Ti106-1	31.05	31.15
TiSt49-1	31.26	31.2
TiSt51-1	31.76	32.08
TiSt52-1	31.38	31.95
TiSt53-1	30.66	30.6

	Ct values	
Stage-2 Samples	Replicate 1	Replicate 2
Be55-2	28.25	28.31
Be57-2	29.01	29.24
Be60-2	28.9	28.94
BeSt1-2	28.4	28.75
BeSt3-2	28.09	28.33
BeSt5-2	28.78	28.75
Ds63-2	29.91	29.89
Ds65-2	28.63	29.04
Ds66-2	29.67	29.89
DsSt11-2	29.69	30.02
DsSt7-2	30.39	30.09
DsSt9-2	29.85	29.83
Gu73-2	29.45	29.36
Gu74-2	29.74	29.56
Gu76-2	30.1	29.52
GuSt21-2	29.69	29.77
GuSt22-2	30.25	30.09
GuSt24-2	29.49	29.72
Pi93-2	31.94	31.99
Pi94-2	31.21	31.15
Pi95-2	29.95	30.19
PiSt37-2	32.4	32.26
PiSt38-2	31.08	31.16
PiSt39-2	30.74	31.12
Sa100-2	32.66	32.93
Sa101-2	31.89	31.88
Sa102-2	32.03	32.04
SaSt43-2	31.15	31.24
SaSt46-2	31.16	31.22
SaSt48-2	32.2	31.88
Ti104-2	31.79	31.79
Ti105-2	30.84	31.56
Ti106-2	30.89	31.07
TiSt50-2	31.72	32.11
TiSt52-2	31.61	32.12
TiSt53-2	31.41	31.71

Stage-3 Samples	Replicate 1	Replicate 2
Be3-4	34.22	33.79
Be3-58	35.02	37.1
Be3-6	33.33	34.14
Sa3-46	36.79	38.99
Sa3-47	36.89	37.22
Sa3-48	36.5	38.86
Ti3-106	39.04	38.45
Ti3-50	N/A	N/A
Ti3-51	35.61	36.26

Stage-4 Samples	Replicate 1	Replicate 2
Be4-1	32.12	33.03
Be4-55	34.54	33.67
Be4-56	32.01	35.46
Sa4-43	35.39	32.63
Sa4-46	35.94	34.63
Sa4-47	34.46	32.97
Ti4-106	35	35.64
Ti4-49	34.06	34.45
Ti4-50	36.69	34.26

D.2.2 Gene *PGSC0003DMG400017087*, putatively encoding late blight resistance protein homologue r1a-10

	Ct values	
Stage-1 Samples	Replicate 1	Replicate 2
Be55-1	30.05	29.93
Be56-1	30.85	29.97
Be57-1	29.36	29.16
BeSt1-1	29.92	29.71
BeSt3-1	28.22	27.67
BeSt5-1	27.87	27.54
Ds61-1	27.04	27.06
Ds64-1	26.65	26.61
Ds66-1	26.63	27.08
DsSt11-1	26.83	26.99
DsSt7-1	29.52	28.39
DsSt9-1	26.74	26.7
Gu73-1	32.84	33.08
Gu74-1	30.54	30.48
Gu76-1	31.77	31.43
GuSt21-1	31.75	31.67
GuSt22-1	30.77	33.07
GuSt24-1	30.19	30.34
Pi93-1	28.72	28.09
Pi94-1	29.09	28.42
Pi95-1	29.89	30.02
PiSt37-1	29.57	30.06
PiSt38-1	29.25	31.7
PiSt41-1	30.38	30.52
Sa100-1	36.05	36.09
Sa101-1	33.67	33.04
Sa102-1	32.85	31.85
SaSt43-1	32.83	33.52
SaSt47-1	33.53	32.09
SaSt48-1	31.77	31.14
Ti105-1	31.6	31.61
Ti106-1	26.82	26.67
TiSt49-1	31.09	31.32
TiSt51-1	29.73	29.45
TiSt52-1	27.5	28.59
TiSt53-1	34.19	36.52

	Ct values	
Stage-2 Samples	Replicate 1	Replicate 2
Be55-2	26.7	26.71
Be57-2	27.41	28.21
Be60-2	28.98	29.59
BeSt1-2	24.78	25.26
BeSt3-2	25.33	24.74
BeSt5-2	24.46	24.49
Ds63-2	24.46	24.55
Ds65-2	23.73	23.78
Ds66-2	24.58	25
DsSt11-2	25.1	25.49
DsSt7-2	25.68	25.71
DsSt9-2	24.17	24.28
Gu73-2	29.52	28.61
Gu74-2	29.46	28.52
Gu76-2	28.63	27.96
GuSt21-2	25.12	24.82
GuSt22-2	27.18	26.62
GuSt24-2	25.51	25.23
Pi93-2	28.07	28.05
Pi94-2	25.31	25.31
Pi95-2	26.82	26.58
PiSt37-2	27.03	27.05
PiSt38-2	25.61	25.4
PiSt39-2	27.22	27.37
Sa100-2	34.43	34.36
Sa101-2	29	28.87
Sa102-2	30.14	30.07
SaSt43-2	28.64	28.73
SaSt46-2	29.19	28.89
SaSt48-2	28.27	28.42
Ti104-2	23.73	23.67
Ti105-2	25.87	26.08
Ti106-2	25.35	25.13
TiSt50-2	24.78	25.01
TiSt52-2	25.47	25.47
TiSt53-2	26.35	26.52

Stage-3 Samples	Replicate 1	Replicate 2
Be3-4	26.36	26.1
Be3-58	29.67	29.25
Be3-6	27.23	26.68
Sa3-46	N/A	31.43
Sa3-47	31.08	N/A
Sa3-48	N/A	31.01
Ti3-106	29.11	30.56
Ti3-50	27.94	30.49
Ti3-51	30.14	31.4

Stage-4 Samples	Replicate 1	Replicate 2
Be4-1	29.78	29.24
Be4-55	33.6	33.61
Be4-56	32.59	32.05
Sa4-43	35.65	34.8
Sa4-46	30.26	30.5
Sa4-47	33.64	33.34
Ti4-106	29.03	28.92
Ti4-49	29.3	29.65
Ti4-50	27.48	27.84

D.2.3 Gene *PGSC0003DMG400007385* gene, putatively encoding disease resistant protein At4g27190

	Ct values	
Stage-1 Samples	Replicate 1	Replicate 2
Be55-1	26.31	26.17
Be56-1	28.62	28.29
Be57-1	26.95	26.6
BeSt1-1	27.97	28.22
BeSt3-1	26.9	26.73
BeSt5-1	26.6	26.48
Ds61-1	25.4	25.93
Ds64-1	25.33	26.25
Ds66-1	24.52	25.89
DsSt11-1	25.87	26.66
DsSt7-1	23.79	24.87
DsSt9-1	23.19	24.21
Gu73-1	33.34	31.82
Gu74-1	34.07	31.86
Gu76-1	32.6	31.19
GuSt21-1	33.1	32.04
GuSt22-1	33.02	32.16
GuSt24-1	31.42	30.98
Pi93-1	25.44	24.5
Pi94-1	26.11	25.2
Pi95-1	25.27	23.5
PiSt37-1	27.05	26.03
PiSt38-1	26.98	26.2
PiSt41-1	25.57	24.62
Sa100-1	35.75	34.1
Sa101-1	32.19	30.23
Sa102-1	31.77	30.8
SaSt43-1	32.69	31.45
SaSt47-1	33.86	32.79
SaSt48-1	32.92	31.87
Ti105-1	33.03	32
Ti106-1	34.16	32.39
TiSt49-1	33.18	32.2
TiSt51-1	34.31	33.14
TiSt52-1	33.95	33.6
TiSt53-1	33.01	31.89

Stage-2 Samples	Replicate 1	Replicate 2
Be55-2	25.15	25.18
Be57-2	23.99	24.23
Be60-2	27.02	26.97
BeSt1-2	27.6	27.11
BeSt3-2	26.22	25.74
BeSt5-2	26	25.84
Ds63-2	25.55	26.03
Ds65-2	25.32	25.84
Ds66-2	26.4	27.61
DsSt11-2	26.95	27.12
DsSt7-2	28.06	27.53
DsSt9-2	26.17	25.99
Gu73-2	33.39	32.91
Gu74-2	35.15	33.95
Gu76-2	34.69	33.19
GuSt21-2	31.46	31.56
GuSt22-2	33.23	33.28
GuSt24-2	33	32.62
Pi93-2	25.08	24.95
Pi94-2	24.52	24.18
Pi95-2	24.83	23.21
PiSt37-2	26.19	26.47
PiSt38-2	26.72	26.98
PiSt39-2	28.09	28.21
Sa100-2	N/A	N/A
Sa101-2	33.49	33.87
Sa102-2	34.29	33.66
SaSt43-2	33.7	33.73
SaSt46-2	33.32	32.81
SaSt48-2	32.27	32.35
Ti104-2	35.21	35.03
Ti105-2	35.03	34.12
Ti106-2	34.89	34.38
TiSt50-2	33.59	33.56
TiSt52-2	34.36	35.03
TiSt53-2	34.84	35.53

Stage-3 Samples	Replicate 1	Replicate 2
Be3-4	25.78	26.03
Be3-58	26.24	26.19
Be3-6	26.01	26.12
Sa3-46	31.42	31.28
Sa3-47	30.71	30.4
Sa3-48	30.07	30.14
Ti3-106	35.78	34.38
Ti3-50	33.97	35.22
Ti3-51	33.79	34.92

Stage-4 Samples	Replicate 1	Replicate 2
Be4-1	26.26	26.24
Be4-55	27.72	27.94
Be4-56	27.18	27.25
Sa4-43	N/A	33.31
Sa4-46	N/A	32.33
Sa4-47	N/A	34.18
Ti4-106	33.06	33.9
Ti4-49	33.17	33.78
Ti4-50	34.05	35.13

D.2.4 Gene *PGSC0003DMG400010612* gene, putatively coding for disease resistant protein rpp13

	Ct values	
Stage-1 Samples	Replicate 1	Replicate 2
Be55-1	25.89	25.67
Be56-1	27.11	27.01
Be57-1	26.37	26.32
BeSt1-1	27.68	27.83
BeSt3-1	26.26	26.38
BeST5-1	26.24	26.18
Ds61-1	29.82	29.96
Ds64-1	30.44	30.92
Ds66-1	30.36	30.44
DsSt11-1	32.05	32.84
DsSt7-1	30.32	30.56
DsSt9-1	29.76	29.68
Gu73-1	26.42	27.22
Gu74-1	26.24	27.16
Gu76-1	26.18	26.97
GuSt21-1	27.18	28.49
GuSt22-1	27.81	29.12
GuSt24-1	28.11	29.35
Pi93-1	32.86	33.01
Pi94-1	33.25	34.02
Pi95-1	34.06	33.72
PiSt37-1	33.03	33.25
PiSt38-1	33.36	32.68
PiSt41-1	33.71	34.08
Sa100-1	31.24	30.37
Sa101-1	28.18	27.4
Sa102-1	28.46	28.32
SaSt43-1	29.35	28.69
SaSt47-1	30.51	29.99
SaSt48-1	29.63	29.18
Ti105-1	30.52	31.12
Ti106-1	32.46	32.27
TiSt49-1	29.78	30.1
TiSt51-1	31.18	31.82
TiSt52-1	31.44	32.02
TiSt53-1	30.26	30.1

	Ct values	
Stage-2 Samples	Replicate 1	Replicate 2
Be55-2	27.28	25.16
Be57-2	28.44	25.9
Be60-2	29.43	26.99
BeSt1-2	27.13	26.06
BeSt3-2	27.02	25.15
BeSt5-2	27.32	26.01
Ds63-2	31.59	31.16
Ds65-2	31.19	30.53
Ds66-2	32.51	31.45
DsSt11-2	32.55	32.45
DsSt7-2	31.99	32.59
DsSt9-2	31.59	31.62
Gu73-2	28.11	28.65
Gu74-2	28.17	29.03
Gu76-2	27.43	28.21
GuSt21-2	29.26	29.39
GuSt22-2	29.24	29.1
GuSt24-2	27.94	28.72
Pi93-2	35.35	34.71
Pi94-2	35.05	34.14
Pi95-2	35.09	34.47
PiSt37-2	35.81	36.18
PiSt38-2	35.4	35.02
PiSt39-2	35.94	36.6
Sa100-2	38.11	36.55
Sa101-2	30.48	30.79
Sa102-2	30.28	30.61
SaSt43-2	30.25	30.59
SaSt46-2	29.28	29.54
SaSt48-2	29.49	29.58
Ti104-2	32.98	32.58
Ti105-2	30.83	31.31
Ti106-2	32.6	32.94
TiSt50-2	30.75	31.19
TiSt52-2	31.57	31.48
TiSt53-2	33.12	33.71

Stage-3 Samples	Replicate 1	Replicate 2
Be3-4	23.15	23.13
Be3-58	25.09	25.06
Be3-6	25.01	24.88
Sa3-46	26.97	26.92
Sa3-47	26.93	27.05
Sa3-48	26.23	26.53
Ti3-106	33.12	34.95
Ti3-50	32.52	33.22
Ti3-51	34.34	33.03

Stage-4 Samples	Replicate 1	Replicate 2
Be4-1	25.37	25.55
Be4-55	28.28	28.33
Be4-56	27.59	27.33
Sa4-43	29.63	29.79
Sa4-46	26.86	27.38
Sa4-47	28.5	28.59
Ti4-106	33.08	32.95
Ti4-49	32.57	33.28
Ti4-50	32.76	32.52

D.2.5 Gene *PGSC0003DMG400013405*, putatively encoding myb-related protein myb4

	Ct values	
Stage-1 Samples	Replicate 1	Replicate 2
Be55-1	31.47	31.41
Be56-1	33.7	31.4
Be57-1	32.38	30.73
BeSt1-1	33.33	33.49
BeSt3-1	34.58	32.01
BeSt5-1	31.17	28.82
Ds61-1	30.14	30.6
Ds64-1	32.04	33.51
Ds66-1	33.05	33.57
DsSt11-1	33.21	32.36
DsSt7-1	34.3	34.79
DsSt9-1	32.09	32.21
Gu73-1	33.35	33.92
Gu74-1	33.29	34.2
Gu76-1	31.33	32.64
GuSt21-1	33.08	33.82
GuSt22-1	33.28	33.41
GuSt24-1	33.58	35.65
Pi93-1	31.84	32
Pi94-1	32.14	33.24
Pi95-1	33.44	33.18
PiSt37-1	35.37	35
PiSt38-1	32.73	33.41
PiSt41-1	33.45	33.52
Sa100-1	33.84	33.79
Sa101-1	30.8	30.07
Sa102-1	29.49	29.37
SaSt43-1	31.6	31.02
SaSt47-1	31.52	31.17
SaSt48-1	30.44	30.51
Ti105-1	31.26	31.3
Ti106-1	30.29	30.92
TiSt49-1	30.02	29.32
TiSt51-1	31.84	31.84
TiSt52-1	31.35	31.78
TiSt53-1	34.1	32.88

	Ct values	
Stage-2 Samples	Replicate 1	Replicate 2
Be55-2	33.29	32.93
Be57-2	34.43	34.55
Be60-2	35.05	35.13
BeSt1-2	33.31	33.3
BeSt3-2	32.16	32.3
BeSt5-2	32.48	32.53
Ds63-2	33.89	34.86
Ds65-2	33.73	33.86
Ds66-2	34.35	35.27
DsSt11-2	34.31	35.17
DsSt7-2	35.23	35.53
DsSt9-2	35.21	34.74
Gu73-2	35.75	35.11
Gu74-2	34.96	34.12
Gu76-2	32.66	32.34
GuSt21-2	35.51	35.36
GuSt22-2	34.01	33.14
GuSt24-2	34.03	34.07
Pi93-2	33.45	33.74
Pi94-2	33.33	33.39
Pi95-2	34.19	33.84
PiSt37-2	35.7	35.07
PiSt38-2	35.78	35.33
PiSt39-2	34.53	34.32
Sa100-2	38.66	37.28
Sa101-2	31.2	30.69
Sa102-2	31.44	31.61
SaSt43-2	32.28	32.51
SaSt46-2	30.66	30.76
SaSt48-2	30.8	30.78
Ti104-2	30.81	30.47
Ti105-2	31.46	30.89
Ti106-2	32.13	31.69
TiSt50-2	33.03	31.74
TiSt52-2	31.85	30.92
TiSt53-2	32.92	32.83

Stage-3 Samples	Replicate 1	Replicate 2
Be3-4	31.29	30.98
Be3-58	34.95	34.43
Be3-6	34.72	33.14
Sa3-46	31.48	31.38
Sa3-47	31.24	31.26
Sa3-48	30.38	30.63
Ti3-106	33.57	33.9
Ti3-50	34.87	35.04
Ti3-51	35.26	35.6

Stage-4 Samples	Replicate 1	Replicate 2
Be4-1	34.08	33.85
Be4-55	35.22	35.51
Be4-56	35.83	35.15
Sa4-43	35.15	35.1
Sa4-46	31.27	31.07
Sa4-47	34.04	34.02
Ti4-106	33.31	33.24
Ti4-49	33.97	33.19
Ti4-50	33.15	32.99

D.2.6 Gene *PGSC0003DMG400000655*, putatively coding serine threonine-protein phosphatase-7

	Ct values	
Stage-1 Samples	Replicate 1	Replicate 2
Be55-1	24.04	23.93
Be56-1	27.63	27.62
Be57-1	27.13	27.34
BeSt1-1	27.53	27.9
BeSt3-1	27.48	27.19
BeSt5-1	28.13	27.69
Ds61-1	28.96	28.95
Ds64-1	29.3	29.15
Ds66-1	29.75	29.83
DsSt11-1	27.89	27.08
DsSt7-1	28.21	28.52
DsSt9-1	28.77	28.32
Gu73-1	34.62	34.62
Gu74-1	32.66	33.12
Gu76-1	36.33	36.47
GuSt21-1	34.3	35.02
GuSt22-1	34.86	35.28
GuSt24-1	33.43	35.07
Pi93-1	28.75	28.34
Pi94-1	28.61	28.29
Pi95-1	28.22	28.29
PiSt37-1	29.76	28.83
PiSt38-1	29.32	28.8
PiSt41-1	30.71	29.44
Sa100-1	N/A	N/A
Sa101-1	N/A	N/A
Sa102-1	38.81	38.87
SaSt43-1	39.91	38.67
SaSt47-1	N/A	N/A
SaSt48-1	37.30	37.47
Ti105-1	N/A	N/A
Ti106-1	N/A	N/A
TiSt49-1	N/A	N/A
TiSt51-1	N/A	N/A
TiSt52-1	N/A	N/A
TiSt53-1	N/A	N/A

	Ct values	
Stage-2 Samples	Replicate 1	Replicate 2
Be55-2	23.83	25.18
Be57-2	26.56	26.83
Be60-2	27.39	27.9
BeSt1-2	25.91	25.66
BeSt3-2	26.08	25.46
BeSt5-2	26.71	26.94
Ds63-2	29.57	28.44
Ds65-2	26.89	28.1
Ds66-2	28.05	29.41
DsSt11-2	28.3	29.4
DsSt7-2	28.85	29.56
DsSt9-2	28.15	28.39
Gu73-2	36.54	38.56
Gu74-2	34.35	34.05
Gu76-2	36.7	N/A
GuSt21-2	36.56	34.37
GuSt22-2	35.19	33.06
GuSt24-2	35.01	31.65
Pi93-2	29.43	29.66
Pi94-2	27.52	27.68
Pi95-2	27.19	27.46
PiSt37-2	32.39	31.81
PiSt38-2	29.69	29.47
PiSt39-2	30.39	30.54
Sa101-2	N/A	36.25
Sa102-2	N/A	37.61
SaSt48-2	37.6	N/A
SaSt43-2	N/A	N/A
SaSt46-2	N/A	N/A
SaSt48-2	N/A	N/A
Ti104-2	38.52	N/A
Ti105-2	36.93	37.07
Ti106-2	38	N/A
TiSt50-2	36.06	38.6
TiSt52-2	36.35	36.49
TiSt53-2	N/A	N/A

Stage-3 Samples	Replicate 1	Replicate 2
Be3-4	31.46	31.2
Be3-58	31.18	30.81
Be3-6	28.8	29.23
Sa3-46	N/A	N/A
Sa3-47	N/A	N/A
Sa3-48	N/A	N/A
Ti3-106	N/A	N/A
Ti3-50	N/A	N/A
Ti3-51	N/A	N/A

Stage-4 Samples	Replicate 1	Replicate 2
Be4-1	31.29	31.65
Be4-55	27.42	28.06
Be4-56	32.04	32.33
Sa4-43	N/A	N/A
Sa4-46	N/A	N/A
Sa4-47	N/A	N/A
Ti4-106	N/A	N/A
Ti4-49	N/A	N/A
Ti4-50	N/A	N/A

D.2.7 Gene *PGSC0003DMG400015425*, putatively encoding for sre1a protein

	Ct values	
Stage-1 Samples	Replicate 1	Replicate 2
Be55-1	19.89	20.06
Be56-1	21.08	21.06
Be57-1	21.02	20.83
BeSt1-1	22.2	22.26
BeSt3-1	20.48	20.67
BeSt5-1	21.11	21.37
Ds61-1	22.2	22.35
Ds64-1	22.63	22.68
Ds66-1	23.02	23.21
DsSt11-1	21.36	21.73
DsSt7-1	22.12	22.35
DsSt9-1	21.97	22.17
Gu73-1	21.24	21.65
Gu74-1	20.6	21.01
Gu76-1	21.81	21.87
GuSt21-1	21.06	21.71
GuSt22-1	22.12	22.82
GuSt24-1	23.66	24.48
Pi93-1	21.45	21.44
Pi94-1	21.55	21.49
Pi95-1	21.35	20.81
PiSt37-1	20.86	21.04
PiSt38-1	20.9	20.83
PiSt41-1	21.48	21.44
Sa100-1	23.5	23.79
Sa101-1	20.17	20.34
Sa102-1	20.2	20.45
SaSt43-1	22.27	22.44
SaSt47-1	22.49	22.54
SaSt48-1	23	23.18
Ti105-1	20.01	20.76
Ti106-1	20.3	20.58
TiSt49-1	21.13	21.59
TiSt51-1	22.65	22.3
TiSt52-1	22.56	22.37
TiSt53-1	22.71	22.64

	Ct values	
Stage-2 Samples	Replicate 1	Replicate 2
Be55-2	20.47	22.1
Be57-2	21.44	23
Be60-2	22.24	23.41
BeSt1-2	20.89	22.06
BeSt3-2	20.48	22.21
BeSt5-2	21.28	22.09
Ds63-2	24.64	24.65
Ds65-2	23.39	23.52
Ds66-2	25.1	24.78
DsSt11-2	24.79	25.04
DsSt7-2	25.29	25.48
DsSt9-2	24.51	24.52
Gu73-2	23.68	21.89
Gu74-2	23.63	22.25
Gu76-2	24.51	22.43
GuSt21-2	24.6	23.15
GuSt22-2	24.96	23.96
GuSt24-2	23.29	21.66
Pi93-2	23.61	23.07
Pi94-2	22.17	21.88
Pi95-2	21.89	21.26
PiSt37-2	24.61	24.49
PiSt38-2	24.18	24.03
PiSt39-2	25.53	25.5
Sa100-2	30.43	30.09
Sa101-2	23.76	23.93
Sa102-2	23.59	23.62
SaSt43-2	22.92	23
SaSt46-2	23.09	23.08
SaSt48-2	22.35	22.62
Ti104-2	21.86	22.14
Ti105-2	21.04	21.34
Ti106-2	21.82	21.8
TiSt50-2	22.77	23.1
TiSt52-2	22.54	22.47
TiSt53-2	23.36	23.48

Stage-3 Samples	Replicate 1	Replicate 2
Be3-4	22.25	22.68
Be3-58	22.03	21.84
Be3-6	22.37	22.22
Sa3-46	23.08	N/A
Sa3-47	22.02	22.34
Sa3-48	N/A	N/A
Ti3-106	24.06	24.83
Ti3-50	23.31	24.6
Ti3-51	24.36	25

Stage-4 Samples	Replicate 1	Replicate 2
Be4-1	22.56	22.62
Be4-55	24.69	24.79
Be4-56	24.57	24.57
Sa4-43	26.79	26.63
Sa4-46	22.86	22.83
Sa4-47	24.02	24.12
Ti4-106	24.8	24.95
Ti4-49	24.62	24.58
Ti4-50	25.17	25.13

D.3 Genes putatively involved in thaxtomin A transport

D.3.1 Gene *PGSC0003DMG400014859*, putatively encoding trx1-like protein

	Ct values	
Stage-1 Samples	Replicate 1	Replicate 2
Be55-1	25.06	24.99
Be56-1	26.22	26.01
Be57-1	25.3	25.27
BeSt1-1	27.25	27.17
BeSt3-1	25.67	25.45
BeSt5-1	26.27	26.04
Ds61-1	25.89	25.86
Ds64-1	26.04	26.14
Ds66-1	26.09	26.12
DsSt11-1	25.03	25.06
DsSt7-1	25.18	25.21
DsSt9-1	24.8	24.84
Gu73-1	24.07	24.09
Gu74-1	23.99	23.9
Gu76-1	25.34	25.34
GuSt21-1	24.66	25.11
GuSt22-1	25.16	25.4
GuSt24-1	25.86	26.02
Pi93-1	26.39	25.38
Pi94-1	26.56	25.54
Pi95-1	25.68	24.99
PiSt37-1	26.22	25.29
PiSt38-1	26.33	25.41
PiSt41-1	26.28	25.71
Sa100-1	28.78	28.06
Sa101-1	24.45	24.01
Sa102-1	24.5	24.31
SaSt43-1	26.66	26.23
SaSt47-1	27.16	26.56
SaSt48-1	28.05	27.85
Ti105-1	24.81	24.77
Ti106-1	25.06	24.81
TiSt49-1	25.77	25.54
TiSt51-1	26.43	26.04
TiSt52-1	26.9	26.48
TiSt53-1	26.16	25.87

	Ct values	
Stage-2 Samples	Replicate 1	Replicate 2
Be55-2	26.53	26.22
Be57-2	26.46	26.03
Be60-2	27.79	27.09
BeSt1-2	26.47	27.01
BeSt3-2	26.36	26.62
BeSt5-2	26.01	25.95
Ds63-2	25.98	26.4
Ds65-2	25.65	26.07
Ds66-2	26.3	27.02
DsSt11-2	27.47	27.8
DsSt7-2	28.04	28.54
DsSt9-2	26.64	26.82
Gu73-2	24.93	24.64
Gu74-2	25.33	25.11
Gu76-2	25.24	24.46
GuSt21-2	24.45	25.22
GuSt22-2	26.41	26.55
GuSt24-2	24.64	24.87
Pi93-2	25.32	25.38
Pi94-2	24.25	24.47
Pi95-2	24.16	24.18
PiSt37-2	25.97	26.53
PiSt38-2	26.1	26.14
PiSt39-2	28.19	28.25
Sa100-2	31.27	31.2
Sa101-2	25.27	25.09
Sa102-2	26.12	26.29
SaSt43-2	25.95	26.13
SaSt46-2	26.13	26.31
SaSt48-2	26.65	27.01
Ti104-2	25.72	25.61
Ti105-2	25.34	24.82
Ti106-2	25.06	25.02
TiSt50-2	25.17	25.25
TiSt52-2	25.84	25.6
TiSt53-2	25.78	25.49

Stage-3 Samples	Replicate 1	Replicate 2
Be3-4	25.5	25.58
Be3-58	25.43	25.55
Be3-6	25.29	25.4
Sa3-46	24.96	25.25
Sa3-47	24.36	24.25
Sa3-48	25.08	24.9
Ti3-106	27.36	27.56
Ti3-50	26.64	26.56
Ti3-51	26.55	26.54

Stage-4 Samples	Replicate 1	Replicate 2
Be4-1	26.4	26.37
Be4-55	27.41	27.7
Be4-56	27.72	27.48
Sa4-43	27.98	27.75
Sa4-46	25.27	25.28
Sa4-47	25.69	25.7
Ti4-106	27.11	27.04
Ti4-49	26.22	26.25
Ti4-50	25.92	25.95

D.3.2 Gene *PGSC0003DMG400025578*, putatively encoding trx1-like protein

	Ct values	
Stage-1 Samples	Replicate 1	Replicate 2
Be55-1	23	22.97
Be56-1	24.1	24.16
Be57-1	23.35	23.52
BeSt1-1	25.63	25.61
BeSt3-1	23.71	23.61
BeSt5-1	24.25	24.37
Ds61-1	26.14	25.08
Ds64-1	25.69	25.6
Ds66-1	25.48	25.4
DsSt11-1	24.2	24.18
DsSt7-1	24.47	24.27
DsSt9-1	23.93	23.92
Gu73-1	23	23.45
Gu74-1	22.72	23.06
Gu76-1	23.94	24.12
GuSt21-1	23.53	24.5
GuSt22-1	24.34	25.19
GuSt24-1	25.19	25.85
Pi93-1	23.94	23.91
Pi94-1	24.83	24.9
Pi95-1	23.44	23.46
PiSt37-1	24.48	24.65
PiSt38-1	24.28	24.52
PiSt41-1	24.01	24.23
Sa100-1	27.17	26.84
Sa101-1	22.72	22.9
Sa102-1	22.73	22.73
SaSt43-1	25.15	25.1
SaSt47-1	25.24	25.51
SaSt48-1	25.96	26.06
Ti105-1	24.13	22.93
Ti106-1	24.6	24.44
TiSt49-1	24.66	24.2
TiSt51-1	24.56	23.83
TiSt52-1	25.04	24.28
TiSt53-1	24.98	23.97

	Ct values	
Stage-2 Samples	Replicate 1	Replicate 2
Be55-2	26.71	26.62
Be57-2	26.19	28.7
Be60-2	27.88	27.83
BeSt1-2	27.29	27.38
BeSt3-2	28.06	27.88
BeSt5-2	26.46	26.47
Ds63-2	27.86	27.84
Ds65-2	27.68	27.63
Ds66-2	29.8	29.83
DsSt11-2	29.08	28.89
DsSt7-2	N/A	30.8
DsSt9-2	28.81	28.4
Gu73-2	26.47	26.44
Gu74-2	26.4	26.36
Gu76-2	27.65	27.41
GuSt21-2	27.83	27.59
GuSt22-2	27.79	27.87
GuSt24-2	26.93	26.84
Pi93-2	26.59	24.86
Pi94-2	25.71	23.73
Pi95-2	25.28	23.43
PiSt37-2	28.86	27.13
PiSt38-2	27.31	25.89
PiSt39-2	29.65	27.86
Sa100-2	31.25	31.26
Sa101-2	24.59	24.65
Sa102-2	25.22	25.24
SaSt43-2	25.01	25.04
SaSt46-2	25.23	25.57
SaSt48-2	25.73	25.53
Ti104-2	24.01	24.11
Ti105-2	23.15	23.42
Ti106-2	24.25	24.36
TiSt50-2	24.11	24.01
TiSt52-2	23.81	24.23
TiSt53-2	24.05	24.44

Stage-3 Samples	Replicate 1	Replicate 2
Be3-4	24.23	24.39
Be3-58	24.76	24.4
Be3-6	24.38	24.26
Sa3-46	24.2	24.29
Sa3-47	23.55	23.46
Sa3-48	23.89	23.97
Ti3-106	27.14	27.23
Ti3-50	25.9	25.98
Ti3-51	26.48	26.55

Stage-4 Samples	Replicate 1	Replicate 2
Be4-1	24.86	24.85
Be4-55	27.45	27.63
Be4-56	27.26	27.29
Sa4-43	26.82	27.39
Sa4-46	24.17	24.13
Sa4-47	24.75	24.93
Ti4-106	26.62	26.86
Ti4-49	25.02	25.16
Ti4-50	24.61	24.48

D.3.3 Gene *PGSC0003DMG402017989*, putatively encoding cyclic nucleotide-gated ion channel 1-like protein

	Ct values	
Stage-1 Samples	Replicate 1	Replicate 2
Be55-1	24.5	23.21
Be56-1	27.31	26.02
Be57-1	27.2	25.19
BeSt1-1	27.05	25.3
BeSt3-1	25.04	23.86
BeSt5-1	24.54	24.34
Ds61-1	25.04	25.46
Ds64-1	25.51	25.7
Ds66-1	26.26	26.25
DsSt11-1	25.31	25.84
DsSt7-1	27.37	27.34
DsSt9-1	24.96	25.36
Gu73-1	26.85	27.16
Gu74-1	26.37	26.28
Gu76-1	27.49	27.69
GuSt21-1	26.55	26.44
GuSt22-1	26.29	26.19
GuSt24-1	26.04	26.58
Pi93-1	25.85	26.07
Pi94-1	25.84	25.18
Pi95-1	27.94	27.83
PiSt37-1	25.82	25.72
PiSt38-1	23.91	24.39
PiSt41-1	26.71	26.96
Sa100-1	N/A	28.06
Sa101-1	25.49	25.33
Sa102-1	25.33	25.41
SaSt43-1	24.66	24.47
SaSt47-1	27.38	27.42
SaSt48-1	26.3	26.23
Ti105-1	26.03	27.31
Ti106-1	24.57	21.21
TiSt49-1	23.71	22.9
TiSt51-1	24.69	23.91
TiSt52-1	25.19	24.47
TiSt53-1	25.92	23.82

	Ct values	
Stage-2 Samples	Replicate 1	Replicate 2
Be55-2	25.17	25.61
Be57-2	25.01	25.57
Be60-2	24.84	25.56
BeSt1-2	22.29	23.21
BeSt3-2	22.07	23.51
BeSt5-2	21.71	22.21
Ds63-2	23.36	23.49
Ds65-2	23.1	23.17
Ds66-2	23.31	23.12
DsSt11-2	23.2	23.65
DsSt7-2	24.11	25.05
DsSt9-2	23.22	23.96
Gu73-2	27.32	26.01
Gu74-2	26.87	25.67
Gu76-2	26.39	25.01
GuSt21-2	24.86	23.43
GuSt22-2	24.95	23.62
GuSt24-2	24.14	23.1
Pi93-2	24.06	24
Pi94-2	22.14	22.15
Pi95-2	23.67	23.71
PiSt37-2	22.12	22.32
PiSt38-2	22.04	22.11
PiSt39-2	25.41	25.92
Sa100-2	27.44	28.08
Sa101-2	21.99	22.79
Sa102-2	23.84	24.24
SaSt43-2	23.9	23.26
SaSt46-2	22.43	22.96
SaSt48-2	23.02	23.11
Ti104-2	20.92	21.76
Ti105-2	22.55	23.32
Ti106-2	22.89	23.86
TiSt50-2	22.52	22.98
TiSt52-2	23.07	23.44
TiSt53-2	23.23	23.97

Stage-3 Samples	Replicate 1	Replicate 2
Be3-4	25.03	25.21
Be3-58	27.84	28.01
Be3-6	25.51	25.77
Sa3-46	24.75	N/A
Sa3-47	23.77	23.66
Sa3-48	23.93	24.08
Ti3-106	27.26	27.33
Ti3-50	28.43	28.45
Ti3-51	29.36	29.22

Stage-4 Samples	Replicate 1	Replicate 2
Be4-1	26.46	26.38
Be4-55	28.16	28.32
Be4-56	27.69	27.66
Sa4-43	27.27	27.32
Sa4-46	23.55	23.44
Sa4-47	25.83	26
Ti4-106	25.6	25.46
Ti4-49	27.12	27.11
Ti4-50	26.2	26.06

D.4 Gene *PGSC0003DMG400022929*, putatively encoding aminotransferase ALD1

	Ct values	
Stage-1 Samples	Replicate 1	Replicate 2
Be55-1	N/A	N/A
Be56-1	N/A	N/A
Be57-1	N/A	N/A
BeSt1-1	N/A	N/A
BeSt3-1	N/A	N/A
BeSt5-1	N/A	N/A
Ds61-1	29.51	29.97
Ds64-1	28.73	29.28
Ds66-1	29.29	28.84
DsSt11-1	30.59	30.38
DsSt7-1	30.02	29.64
DsSt9-1	28.2	28.09
Gu73-1	31.56	31.87
Gu74-1	31.41	31.37
Gu76-1	32.27	31.8
GuSt21-1	28.91	29.38
GuSt22-1	29.49	29.69
GuSt24-1	29.51	29.43
Pi93-1	30.75	31.18
Pi94-1	31.03	30.8
Pi95-1	31.01	30.22
PiSt37-1	29.98	30.09
PiSt38-1	30	30.56
PiSt41-1	31.57	31.52
Sa100-1	31.49	32.07
Sa101-1	29.13	28.64
Sa102-1	28.45	28.72
SaSt43-1	27.29	27.91
SaSt47-1	29.5	29.89
SaSt48-1	28.55	29.18
Ti105-1	30.06	31.06
Ti106-1	28.91	29.46
TiSt49-1	31.74	32
TiSt51-1	30.69	31.12
TiSt52-1	31.24	31.89
TiSt53-1	31.44	30.56

	Ct values	
Stage-2 Samples	Replicate 1	Replicate 2
Be55-2	33.45	N/A
Be57-2	34.51	35.47
Be60-2	35.01	N/A
BeSt1-2	31.4	31.5
BeSt3-2	29.55	29.38
BeSt5-2	28.77	28.6
Ds63-2	N/A	33.51
Ds65-2	30.15	30.32
Ds66-2	32.07	31.06
DsSt11-2	32.01	30.92
DsSt7-2	35.04	32.35
DsSt9-2	32.46	30.04
Gu73-2	33.82	33
Gu74-2	34.39	36.36
Gu76-2	N/A	34.43
GuSt21-2	N/A	29.1
GuSt22-2	28.81	30.07
GuSt24-2	29.24	30.17
Pi93-2	35.02	30.89
Pi94-2	32.28	29.99
Pi95-2	32.4	30.19
PiSt37-2	31.56	30.1
PiSt38-2	31.15	31.13
PiSt39-2	33.23	35.42
Sa100-2	36.54	33.56
Sa101-2	30.06	28.61
Sa102-2	32.34	32.12
SaSt43-2	28.17	28.72
SaSt46-2	33.29	29.57
SaSt48-2	30.03	29.72
Ti104-2	27.14	27.45
Ti105-2	29.35	29.74
Ti106-2	28.56	29.08
TiSt50-2	29.04	31.57
TiSt52-2	30.67	31.02
TiSt53-2	30.24	30.49

Stage-3 Samples	Replicate 1	Replicate 2
Be3-4	29.99	29.78
Be3-58	29.87	30.92
Be3-6	32.23	31.86
Sa3-46	29.87	29.66
Sa3-47	32.14	32.25
Sa3-48	31.31	31.09
Ti3-106	31.63	33.1
Ti3-50	30.57	28.34
Ti3-51	31.23	31.48

Stage-4 Samples	Replicate 1	Replicate 2
Be4-1	N/A	34.15
Be4-55	29.9	29.52
Be4-56	30.17	30.11
Sa4-43	32.42	33.38
Sa4-46	29.63	29.42
Sa4-47	29.23	29.29
Ti4-106	28.55	30.17
Ti4-49	27.53	27.25
Ti4-50	29.55	30.05

D.5 Genes involved in auxin signaling

D.5.1 Gene *PGSC0003DMG401008875*, with predicted function of ATP binding cassette transporter B family (ABCB)

	Ct values	
Stage-1 Samples	Replicate 1	Replicate 2
Be55-1	26.01	26.66
Be56-1	27.45	27.47
Be57-1	26.53	27.25
BeSt1-1	28.2	28.51
BeSt3-1	26.78	26.99
BeSt5-1	28.01	27.91
Ds61-1	27.34	27.12
Ds64-1	27.79	27.14
Ds66-1	28.83	27.42
DsSt11-1	25.84	25.85
DsSt7-1	26.73	26.37
DsSt9-1	26.11	25.96
Gu73-1	26.03	26.09
Gu74-1	26.19	25.38
Gu76-1	27.57	26.19
GuSt21-1	26.75	26.47
GuSt22-1	27.33	26.92
GuSt24-1	27.32	28.22
Pi93-1	27.04	25.49
Pi94-1	26.06	26
Pi95-1	26.06	25.67
PiSt37-1	25.76	26.11
PiSt38-1	25.62	26.2
PiSt41-1	27.31	27.18
Sa100-1	29.9	30.37
Sa101-1	26.11	27.15
Sa102-1	25.95	26.56
SaSt43-1	27.85	28.16
SaSt47-1	28.74	29.09
SaSt48-1	29.12	29.83
Ti105-1	25.13	25.69
Ti106-1	24.17	25.29
TiSt49-1	26.27	26.37
TiSt51-1	26.38	26.62
TiSt52-1	26.49	26.82
TiSt53-1	27.4	27.22

	Ct values	
Stage-2 Samples	Replicate 1	Replicate 2
Be55-2	28.76	26.35
Be57-2	29.39	27.06
Be60-2	27.18	28.25
BeSt1-2	28.1	27.17
BeSt3-2	29.72	29.09
BeSt5-2	26.97	27.83
Ds63-2	28.02	28.91
Ds65-2	27.35	28.54
Ds66-2	29.59	29.55
DsSt11-2	29.19	28.46
DsSt7-2	28.49	30.22
DsSt9-2	27.6	27.02
Gu73-2	25.98	26.6
Gu74-2	26	26.61
Gu76-2	26.78	27.16
GuSt21-2	26.94	27.03
GuSt22-2	27.28	27.19
GuSt24-2	26.38	26.51
Pi93-2	26.18	26.34
Pi94-2	25.05	25.23
Pi95-2	25.32	25.35
PiSt37-2	27.39	27.57
PiSt38-2	27	27.08
PiSt39-2	29.04	29.25
Sa100-2	33.77	32.2
Sa101-2	27.24	26.46
Sa102-2	27.4	26.54
SaSt43-2	27.42	27.18
SaSt46-2	28.39	27.16
SaSt48-2	28.3	27.55
Ti104-2	25.06	25.22
Ti105-2	24.8	25.08
Ti106-2	24.87	24.85
TiSt50-2	25.72	26.02
TiSt52-2	25.21	25.64
TiSt53-2	26.34	26.5

Stage-3 Samples	Replicate 1	Replicate 2
Be3-4	27.17	27.2
Be3-58	26.38	26.4
Be3-6	26.84	26.77
Sa3-46	26.9	27.07
Sa3-47	26.26	26.2
Sa3-48	26.42	26.45
Ti3-106	27.33	27.66
Ti3-50	27.96	28.15
Ti3-51	28.43	28.69

Stage-4 Samples	Replicate 1	Replicate 2
Be4-1	26.17	26.2
Be4-55	27.44	27.46
Be4-56	27.87	28.1
Sa4-43	29.19	29.05
Sa4-46	26.07	26.17
Sa4-47	26.91	26.76
Ti4-106	26.96	26.66
Ti4-49	26.85	26.7
Ti4-50	26.66	26.71

D.5.2 Gene *PGSC0003DMG401020044*, with predicted function of auxin binding protein 1 (ABP1)

	Ct values	
Stage-1 Samples	Replicate 1	Replicate 2
Be55-1	24.96	23.37
Be56-1	25.45	25.18
Be57-1	24.75	24.21
BeSt1-1	25.55	25.85
BeSt3-1	24.35	24.18
BeSt5-1	25.06	25.11
Ds61-1	24.26	24.21
Ds64-1	24.35	24.77
Ds66-1	24.74	24.78
DsSt11-1	23.8	24.04
DsSt7-1	24.4	24.27
DsSt9-1	23.32	23.43
Gu73-1	23.08	23.19
Gu74-1	22.85	22.89
Gu76-1	23.68	23.48
GuSt21-1	24.09	24.15
GuSt22-1	24.52	24.8
GuSt24-1	24.94	25.18
Pi93-1	24.77	23.73
Pi94-1	24.5	24.03
Pi95-1	24.37	23.55
PiSt37-1	25.01	24.92
PiSt38-1	25.06	24.89
PiSt41-1	25.43	25.15
Sa100-1	27.28	26.3
Sa101-1	23.36	23.07
Sa102-1	23.1	22.87
SaSt43-1	25.08	25.01
SaSt47-1	25.44	24.63
SaSt48-1	25.85	25.29
Ti105-1	22.86	23.3
Ti106-1	22.69	23.11
TiSt49-1	24	24.12
TiSt51-1	23.96	24.34
TiSt52-1	24.18	24.45
TiSt53-1	24.93	24.66

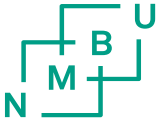
	Ct values	
Stage-2 Samples	Replicate 1	Replicate 2
Be55-2	25.28	25.25
Be57-2	N/A	25.17
Be60-2	26.06	25.93
BeSt1-2	25.1	25.67
BeSt3-2	25.36	25.65
BeSt5-2	24.99	25.18
Ds63-2	26.91	27.53
Ds65-2	26.02	26.83
Ds66-2	27.6	N/A
DsSt11-2	28.03	28.03
DsSt7-2	29.38	28.79
DsSt9-2	26.72	26.77
Gu73-2	25.27	25.57
Gu74-2	25.03	25.36
Gu76-2	25.71	25.83
GuSt21-2	26.01	26.7
GuSt22-2	26.29	26.86
GuSt24-2	25.07	25.6
Pi93-2	26.01	26.09
Pi94-2	24.32	24.61
Pi95-2	24.83	24.85
PiSt37-2	27.84	28.09
PiSt38-2	27.3	27.2
PiSt39-2	28.62	28.76
Sa100-2	32.89	32.5
Sa101-2	25.85	26.01
Sa102-2	25.95	26.19
SaSt43-2	25.94	26.02
SaSt46-2	26.13	26.72
SaSt48-2	26.27	26.69
Ti104-2	24.61	24.8
Ti105-2	23.72	24.21
Ti106-2	N/A	24.78
TiSt50-2	25.12	25.34
TiSt52-2	24.96	25.04
TiSt53-2	25.4	25.65

Stage-3 Samples	Replicate 1	Replicate 2
Be3-4	24.95	24.72
Be3-58	25.11	25.14
Be3-6	24.94	24.78
Sa3-46	25.06	25.14
Sa3-47	24.66	24.35
Sa3-48	24.56	24.77
Ti3-106	27.22	27.48
Ti3-50	26.35	26.66
Ti3-51	26.92	26.96

Stage-4 Samples	Replicate 1	Replicate 2
Be4-1	25.08	24.98
Be4-55	26.33	26.27
Be4-56	26.18	26.14
Sa4-43	27.49	27.29
Sa4-46	24.4	24.4
Sa4-47	25.53	25.4
Ti4-106	26.46	26.33
Ti4-49	25.16	25.16
Ti4-50	25.03	25.2

D. 6 Threshold Cycle values obtained from real-time PCR amplification to detect the presences of candidate genes in the genome

Gene names	DNA samples	Ct values	
		Replicate 1	Replicate 2
PGSC0003DMG400000655	Beate-1.s2	25.03	25.03
PGSC0003DMG400000655	DSxAs-66.s3	26.53	26.27
PGSC0003DMG400000655	Gullauge-74.s2	25.28	25.61
PGSC0003DMG400000655	Pimpernel-95.s2	23.51	23.58
PGSC0003DMG400000655	Saturna-97.s5	24.86	24.56
PGSC0003DMG400000655	Tivoli-53.s2	24.42	24.56
PGSC0003DMG400007385	Beate-1.s2	32.19	32.94
PGSC0003DMG400007385	DSxAs-66.s3	34.41	34.39
PGSC0003DMG400007385	Gullauge-74.s2	34.56	34.32
PGSC0003DMG400007385	Pimpernel-95.s2	32.66	33.24
PGSC0003DMG400007385	Saturna-97.s5	32.17	33.14
PGSC0003DMG400007385	Tivoli-53.s2	32.26	32.41
PGSC0003DMG400010612	Beate-1.s2	30.25	30.44
PGSC0003DMG400010612	DSxAs-66.s3	30.27	30.2
PGSC0003DMG400010612	Gullauge-74.s2	31.51	31.29
PGSC0003DMG400010612	Pimpernel-95.s2	29.32	29.2
PGSC0003DMG400010612	Saturna-97.s5	32.4	32.19
PGSC0003DMG400010612	Tivoli-53.s2	28.02	27.83
PGSC0003DMG400003985	Beate-1.s2	25.78	25.9
PGSC0003DMG400003985	DSxAs-66.s3	27.62	27.46
PGSC0003DMG400003985	Gullauge-74.s2	27.31	27.07
PGSC0003DMG400003985	Pimpernel-95.s2	26.02	25.43
PGSC0003DMG400003985	Saturna-97.s5	25.89	25.36
PGSC0003DMG400003985	Tivoli-53.s2	25.79	25.44



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